SONY

C-181-100-**11** (1)

Cell Sorter

Operator's Guide

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Software Version 1.7

LE-SH800 Series

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The supplied installation disc includes the Operator's Guide for this unit in PDF format.
For more details, see *"Using the PDF Manual" (page 9)*.

- Reproduction or duplication, in whole or part, of the software or operator's guide supplied with the unit, as well as renting or leasing of the software without the authorization of the right holder is prohibited under copyright law.
- Sony assumes no responsibility for damages, loss of income, or any claims from a third party arising out of use of the unit or supplied software.
- For complete terms and conditions of the warranty for the unit, refer to the warranty card included in the package.
- The software supplied with the unit cannot be used with any other units.
- Note that the specifications of the unit and supplied software are subject to change for improvement without prior notice.

Software Revision History

Functions Added in Version 1.7

The following functions have been added in SH800 Software version 1.7.

Support for PEEK sample line

Support added for PEEK sample line that can be detached easily and safely.

For details, see "Sample Line Support" (page 10).

Support for 384-well plates (option)

Sample fluid can now be sorted into 384-well plates using this option.

For details, see "Preparing Collection Tubes" (page 33) and "Sorting" (page 53).

Added sample line exchange to maintenance mode

The sample line can be replaced using a wizard when the unit is in maintenance mode.

For details, see "Running Maintenance Mode" (page 158).

Support for sample line cleaning at startup

Sample line cleaning can be performed during initial setup when starting SH800 Software.

For details, see "Initial Instrument Setup" (page 43).

Improved functionality

The following functions have also been added or expanded.

- Display of remaining sorting time *(page 136 and page 137)*
- Improved accuracy in sorting position adjustment for multi-well plate sorting (four corners of plates) *(page 99)*
- Additional test modes for multi-well plate sorting *(page 100)*
- Added option to skip rinsing of the outside of the sample probe during cleaning using DI water *(page 106)*
- Added multi-well pattern selection buttons for when configuring 8-well strip and multi-well plate sorting *(page 56 and page 59)*

Functions Added in Version 1.6

The following functions have been added in SH800 Software version 1.6.

Support for 130 µm sorting chips on all models

Added support for 130 µm sorting chip, even for 2-way sorting on 96-well plate models.

For details, see "Sorting" (page 53).

Support for AMS (Aerosol Management System) evacuators (SH800Z and SH800ZP)

A S.A.F.E System (evacuator) from Edge Systems LLC can now be connected using a specialized adapter.

For details, see "Connecting an AMS Evacuator (SH800Z and SH800ZP)" (page 213).

[Large Cell] can be selected in all sorting methods

[Large Cell] can now be selected in 8-well strip, slide glass, and 96-well plate sorting methods, in addition to the 2-way sorting method.

Functions Added in Version 1.5.1

The following functions have been added in SH800 Software version 1.5.1.

Supported by the SH800

The following functions were added.

- Index sorting
- Specifying a gate as calculation target for fluorescence compensation
- Data source indication
- Sample empty detection function
- Maintenance mode

Functions Added in Version 1.5

The following functions have been added in SH800 Software version 1.5.

Multi-well plate support (SH800 (96-well plate model))

Sample fluid can be sorted into 6, 12, 24, 48, and 96-well plates.

For details, see "Preparing Collection Tubes" (page 33) and "Sorting" (page 53).

Index sorting

An index sorting function has been added that links the data for sorted events to the individual well in which the events were sorted.

For details, see “Index Sorting” (page 101).

Specifying a gate as calculation target for fluorescence compensation

This function allows you to specify the event population for a gate as a negative or a positive control used for calculating fluorescence compensation.

For details, see “Using a Target Gate in the Gate Hierarchy” (page 80).

Data source indication

A data source component has been added to the Experiment Explorer that indicates the type of sorting collection device for saved data.

For details, see “Data sources” (page 140).

Sample empty detection function

This function detects when the level in the sample tube is too low, and stops data acquisition automatically.

For details about each item, see “[Sample Empty Detection]” (page 123) in the [Advanced Settings] dialog.

Maintenance mode

This function allows an operator to run ethanol cleaning or bleach cleaning without needing to log in to SH800 Software.

For details, see “Running Maintenance Mode” (page 158).

Using the PDF Manual

The Operator’s Guide can be read on a computer with Adobe Reader installed.
You can download Adobe Reader free from the Adobe website.

1. Open the “Operator’s Guide” folder in the installation disc.
2. Select and click on the Operator’s Guide that you want to read.

If you have lost or damaged the installation disc, you can purchase a new one from your Sony distributor.

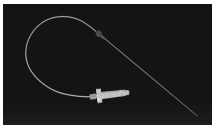
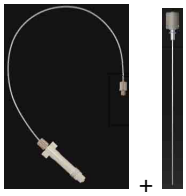
Model Identification

The following model names are used to refer to the various unit models in this document.

Model name	Model[1)]	Description
SH800	LE-SH800AC, BC, CC, DC, EC, FC	Supports collection tube (2-way), 8-well strip, and slide glass
SH800Z	LE-SH800ZA, ZB, ZC, ZD, ZE, ZF, ZG, ZH	
SH800ZP	LE-SH800ZAP, ZBP, ZCP, ZDP, ZEP, ZFP, ZGP, ZHP	Supports collection tube (2-way) and multi-well plate[2) 3)]

1) SH800 Software 1.7 supports both PEEK sample line compatible models and non-PEEK sample line compatible models. See *"Sample Line Support"* (below).
2) SH800 models upgraded to support 96-well plate sorting are equivalent to SH800ZP models.
3) In this document 6, 12, 24, 48, 96, and 384-well plates are collectively referred to as "multi-well plates" or "96-well plates."

Sample Line Support

	PEEK sample line (with probe)	Sample line + sample probe (metallic)
		
PEEK sample line compatible models	Yes	No
Non-PEEK sample line compatible models	No	Yes

Components and Documentation

System Components

The SH800 system comprises the following items.

- Main unit (1)
- Fluidics cart (1)

- Safety Guide (1)
- Operator's Guide (this document) (1)
- Installation disc (Operator's Guide, software) (1)

- Power cord (1)
- PC connection cable (1)
- PEEK sample line (1) and probe adapter (1) combination (PEEK sample line compatible models)
- Sample line (1) and sample probe (1) combination (non-PEEK sample line compatible models)
- Sheath filter (1)
- Sheath filter bypass line (yellow) (1)
- DI water filter bypass line (green) (1)
- DI water filter (1)
- Drip tray (1)
- DI water tank (1)
- DI water tank probe (1)
- Air filter for DI water tank (1)

- Sheath tank (1)
- Ethanol tank (1)
- Ethanol tank probe (1)
- Air filter for ethanol tank (1)
- Waste tank (1)
- Waste tank nozzle (1)
- Air filter for waste tank (1)

- Sheath air line (clear) for fluidics cart (1)
- Sheath line (blue) for fluidics cart (1)
- Ethanol line (yellow) for fluidics cart (1)
- Waste line (red) for fluidics cart (1)
- Cart connection cable (1)

- Sample tube holder (0.5 ml) (1)
- Sample tube holder (1.5 ml) (1)
- Sample tube holder (5 ml) (1)
- Sample tube holder (15 ml) (1)
- Cleaning tube holder (30 ml) (1)

- Collection tube holder (5 ml) (1)
- Collection tube holder (15 ml) (1)

SH800ZP (standard equipment)

- 96-well plate holder (1)
- 96-well PCR plate holder (1)

SH800ZP (options)

- 384-well plate holder (1)
- 384-well PCR adapter (1)

SH800 and SH800Z (options)

- 8-well strips holder (1)
- Slide glass holder (1)

Documentation Structure

The system documentation comprises the following manuals.

- Safety Guide
  Contains safety information, usage precautions, and specifications.
  Read carefully before operating the unit.
- Operator's Guide
  Describes the required preparation and basic operating procedures in order to use the instrument, adjustments and procedures for more advanced use of the instrument, and the configuration items in the SH800 Software windows and dialogs.
  "Chapter 3 Basic Operation" (page 40) provides an overview of the basic procedures, from setting up and calibrating the instrument through to acquiring data, analyzing data, and sorting.
  Refer to the subsequent chapters for detailed information about operating procedures and instrument settings.

Overview

Chapter 1

Main Features

The LE-SH800 series is a benchtop, high-speed, multilaser flow cytometer and cell sorter designed for research laboratory use. It employs a novel, replaceable, microfluidics, cell “sorting chip” that features high reliability and greatly simplifies cell sorting setup. It can be easily and quickly exchanged as needed for quick turnaround between measurements to minimize system downtime and to improve user work flow.

- Flow channels embedded within the chip for precision sheath fluid and sample fluid flow control
- High-uniformity droplet formation
- Automated chip loading and unloading system
- Easy optimization of system sorting and analysis conditions when changing sorting chip

The SH800 interrogates cells in samples using up to four lasers (405 nm, 488 nm, 561 nm, 638 nm), depending on the model. It measures forward scatter, back scatter, and six channels of fluorescent light. The wide range of excitation wavelengths supports the use of a wide variety of common fluorochromes for use in cell analysis.

The SH800 can measure intrinsic properties, such as size and internal complexity using forward scatter and back scatter analysis. It can simultaneously measure a wide range of extrinsic properties using fluorescent light emission analysis.

High-speed electronics allow the operator to specify multiple gates that isolate cells with defined characteristics for further processing. Gates can be applied to provide multi-parameter criteria for real-time identification of selected cell populations at speeds of 100,000 events per second (eps) for analysis and up to 10,000 eps for sorting. The SH800 supports high-speed, two-way sorting to isolate populations for further study or testing.

An array of automated mechanisms is incorporated to enable easy use by non-specialist operators. These systems include:
- Sorting chip loading and alignment
- Sample tube loading
- Collection tube[1)] loading and alignment
- Fluorescence compensation
- Droplet formation, frequency, and calibration
- Sample line and sorting chip cleaning
- Temperature control of sample tube and collection tubes

1) In this document, sample tubes, 8-well strips, slide glass, and multi-well plates used for collecting sorted samples are collectively referred to as "collection tubes."

Manual controls for fluorescence compensation and droplet formation are also supported in SH800 Software.

The hardware user interface is designed to be as simple as possible to use. An LCD monitor on the front of the unit displays status information and notification of error messages.

The SH800 connects to standard 100 V to 240 V AC 50/60 Hz power supplies, and has a 17.5 °C to 27.5 °C (63.5 °F to 81.5 °F) recommended operating temperature range. It communicates with the host computer via the PC connection cable. The SH800 requires the connection of a laboratory compressed-air supply for operation. It can also be supplied using an external air compressor unit. An external fluidics cart, placed under or near the main unit, supplies the main unit with sheath fluid used during testing and ethanol used for cleaning, and collects the waste fluid from the main unit.

A single host computer running SH800 Software controls all aspects of instrument operation, including cell analysis and sorting. The operator uses the software to create experiments for measuring data and to create plots and define statistics for analyzing data. The export functions are fully compliant with Flow Cytometry Standard version 3.0 or 3.1. The software provides quick access to all functions from the main window.

The SH800 has a compact footprint size. The benchtop unit has external dimensions (W × D × H) of 550 × 550 × 720 mm (21 3/4 × 21 3/4 × 28 3/8 in) and weighs 100 kg (220 lb).

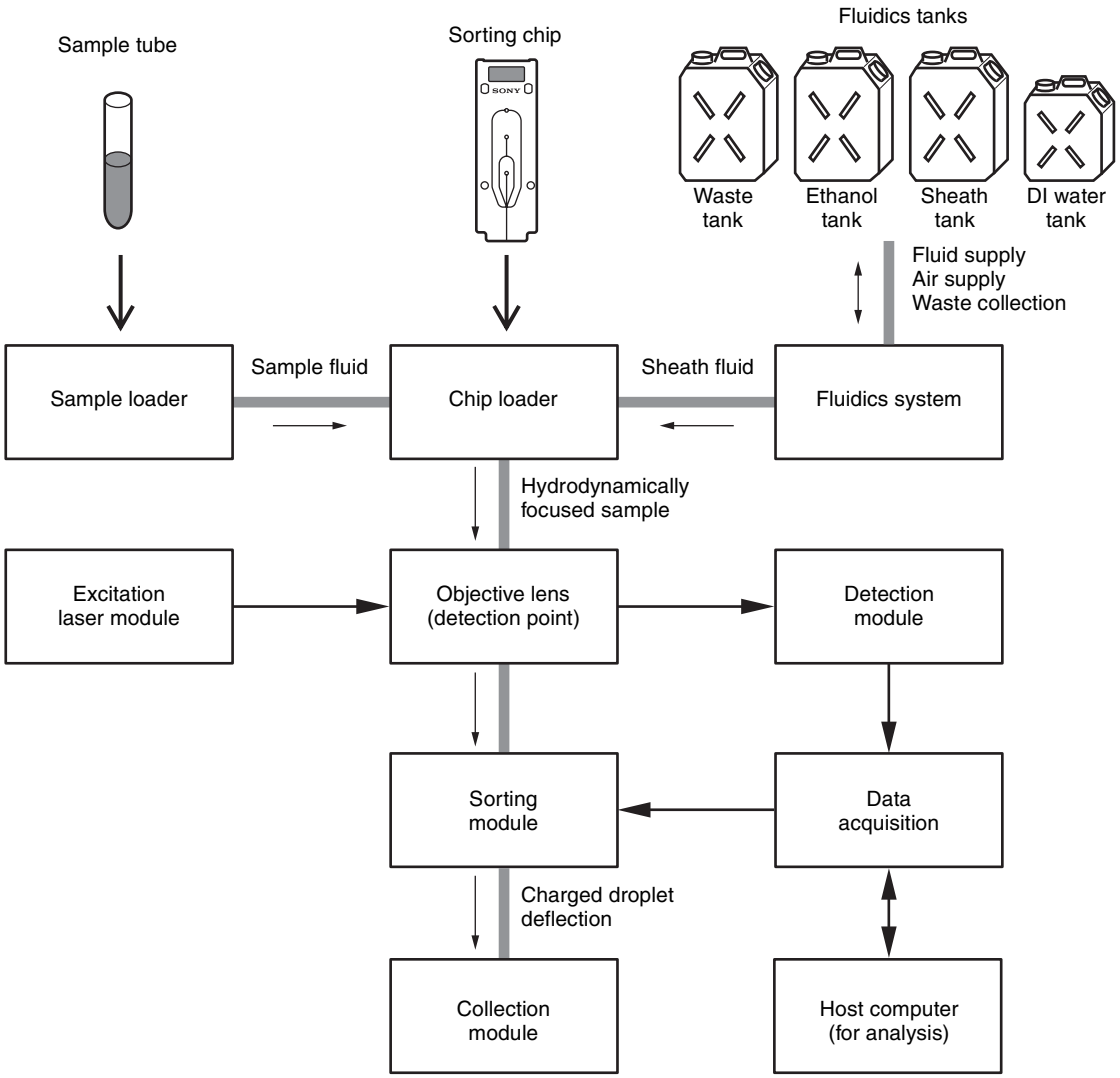
Note

Exposing the main unit or the fluidics lines between the unit and the fluidics cart to drafts from a room air-conditioner may cause droplet formation to become unstable.

Tip

This document describes the basics of flow cytometry as it pertains to understanding how the SH800 cytometer operates. It is not intended as a reference on the subject of flow cytometry. There are many reference sources available, both in print and online, that you should consult for flow cytometry specifics, such as sample preparation, dye selection, analysis techniques, results publication methods, and other topics.

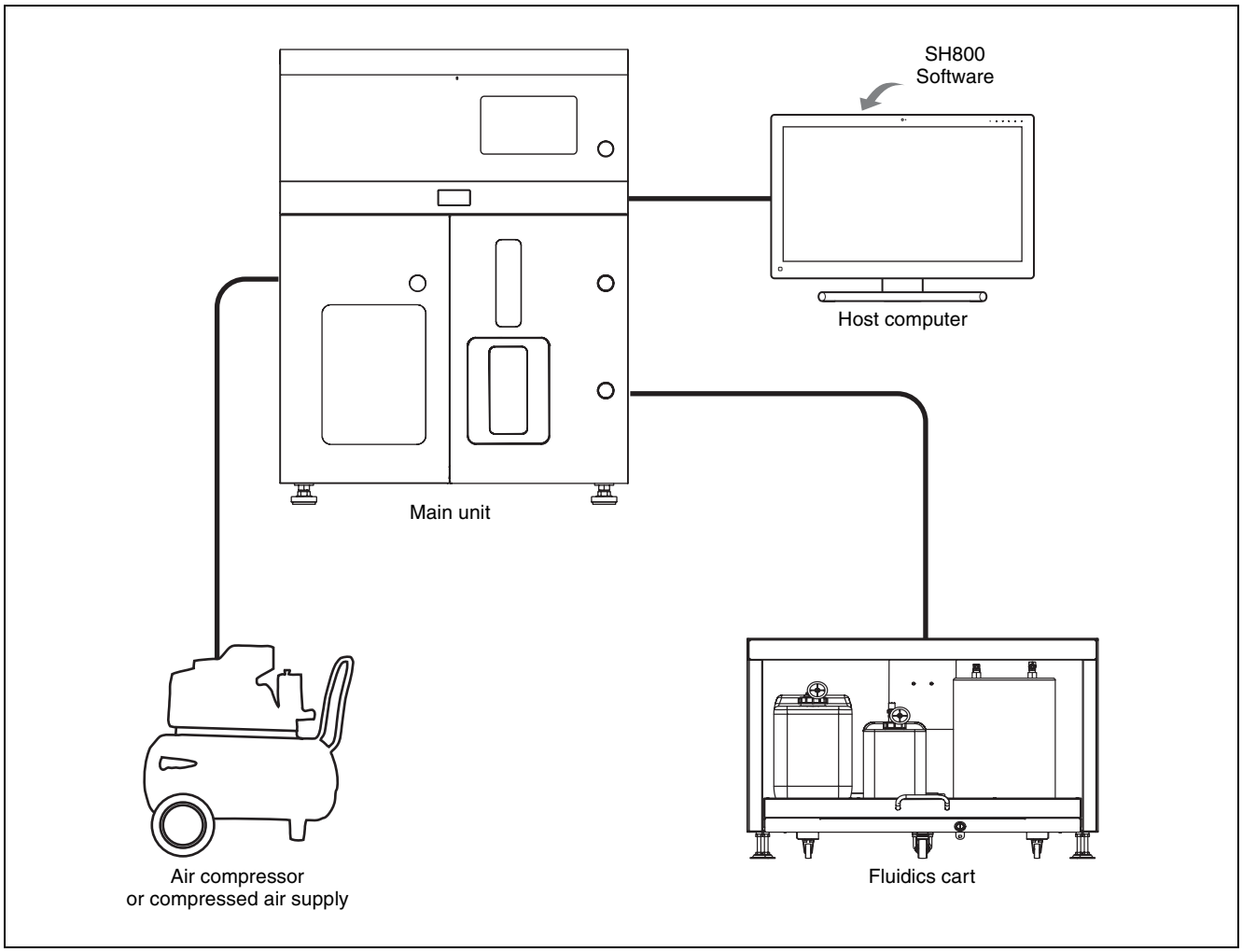
Cell Sorter Block Diagram



• The flow of waste fluid, ethanol, and DI water is not depicted in this diagram. All waste fluid is collected and pumped to the waste tank in the fluidics cart.

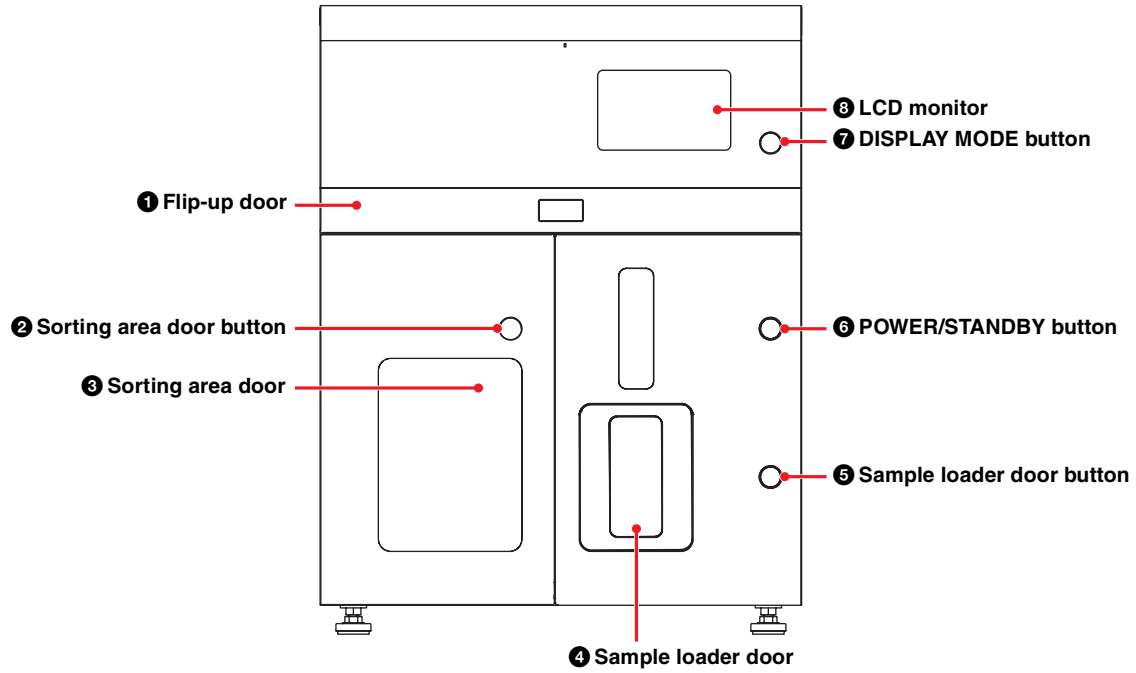
System Configuration

The SH800 system comprises the SH800 main unit, fluidics cart, host computer, and compressed air supply.



Name and Function of Parts

Front Panel



❶ Flip-up door

Provides access to the chip loader for inserting and removing the sorting chip.
The door also provides access to the optical filters, and the sample line and probe for routine maintenance.

Notes

- Make sure to close the flip-up door to run samples.
- The flip-up door opens and closes with assistance from the compressed air supply. Make sure the compressed air supply is turned on and supplying the rated air pressure when operating the door. For safety, a lock function operates to prevent the door from opening and closing when the air supply is turned off.

❷ Sorting area door button

Opens the sorting area door.

❸ Sorting area door

Provides access to the sorting and collection area for loading and unloading collection tubes for sorting. It also provides access for cleaning and routine maintenance.
A safety interlock switch disables operation while the door is open.

Note

A rubber packing seal is used to ensure the integrity of the sorting area door. If the door does not open when the door button is pressed, the door may be sticking to the packing. In this case, open the door by simultaneously pressing the button with one hand while pulling gently on the bottom edge of the door with your other hand.

❹ Sample loader door

Provides access to the sample loader for loading and unloading samples for testing.
A safety interlock switch disables operation while the door is open.

❺ Sample loader door button (OPEN/CLOSE)

Opens and closes the sample loader door.
Once a sample has been lifted into the injection chamber within the sample loader and acquisition started, the button is disabled to prevent opening and closing of the sample loader door.

Note

Observe the following precautions when opening and closing the sample loader door.

- Turn on the power before operating the sample loader door button.
- When opening and closing the door while the power is turned off, make sure the compressed air supply is turned off. Failure to do so may result in damage or injuries.

❻ Power button (POWER/STANDBY)

Switches the SH800 main unit power supply between ON and OFF. When power is ON, press and hold for about two seconds to switch Power OFF.

❼ Display Mode button
Switches the LCD monitor display on/off.

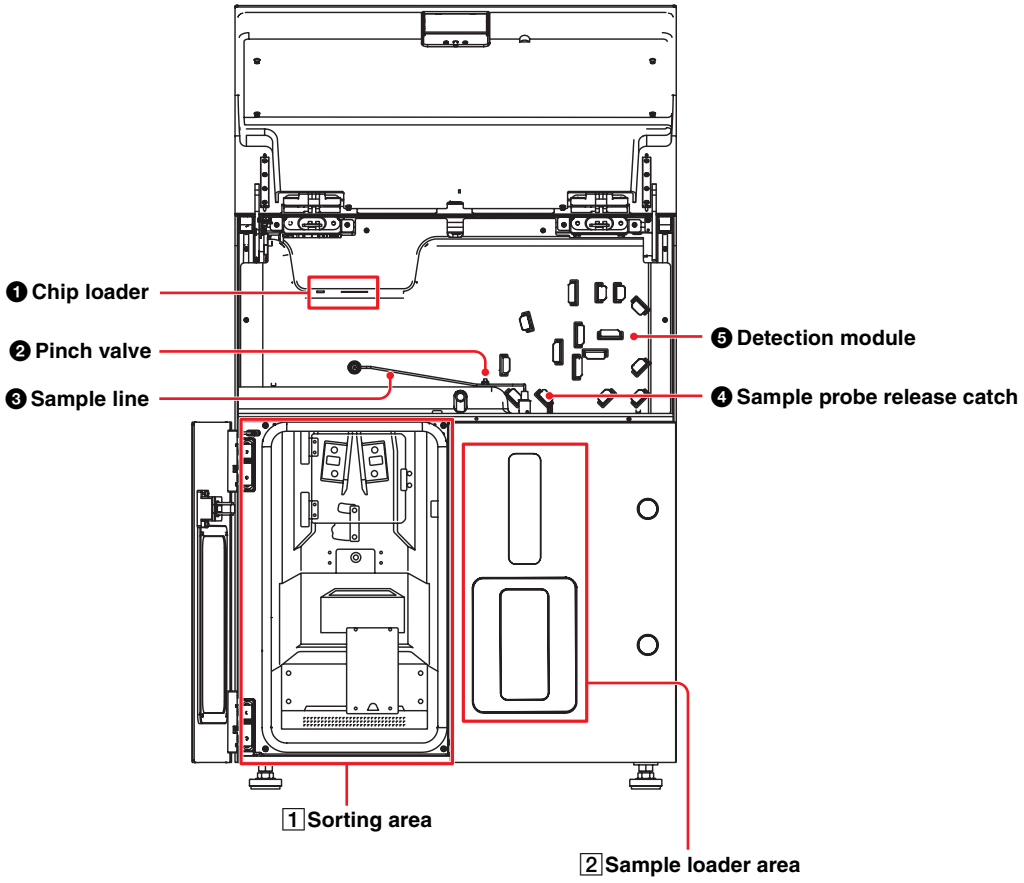
❽ LCD monitor
Displays instrument status information.

For details, see "LCD Monitor" (page 28).

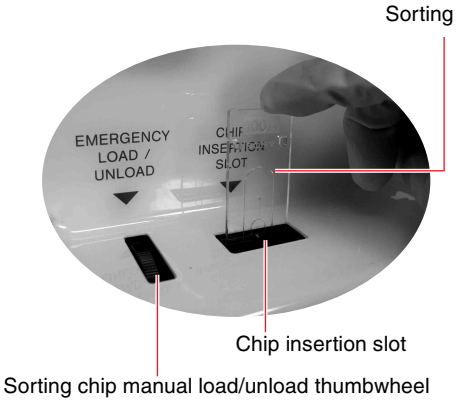
CAUTION
- Do not place any objects in front of the doors.
- Be aware of the door edges when the flip-up door, sorting area door, and fluidics maintenance door are open.

Internal View (Front)

The following illustration depicts the SH800ZP.



❶ Chip loader
Loads and ejects the sorting chip. The chip is inserted in a top-loading slot.



The chip unloading thumbwheel is used to eject the sorting chip if the chip will not unload automatically. For details, see "Ejecting the Sorting Chip Manually" (page 185).

❷ Pinch valve
Controls the flow of sample fluid between the sample probe and the sorting chip.

❸ Sample line
Transports the sample fluid from the sample loader tube to the sorting chip. The PEEK sample line combines the sample line and sample probe in a single component. The sample line and probe are maintenance items.

Notes

- Make sure the sample line is fully inserted into the pinch valve. Failure to do so may result in fluid leakage.
- Touching the sample line will directly affect the chip alignment. If you touch the sample line after the chip alignment has been adjusted, the chip must be realigned using the chip alignment function.
- Always leave the sample line connected when the unit is not in use for protection against dust.

For details, see "Changing the PEEK Sample Line (PEEK sample line compatible models)" (page 169) or "Changing the Sample Line and Probe (Non-PEEK sample line compatible models)" (page 172).

❹ Sample probe release catch

Used when removing the sample probe or probe adapter.

For details, see "Changing the Sample Line and Probe (Non-PEEK sample line compatible models)" (page 172) or "Changing the Probe Adapter (PEEK sample line compatible models)" (page 175).

❺ Detection module

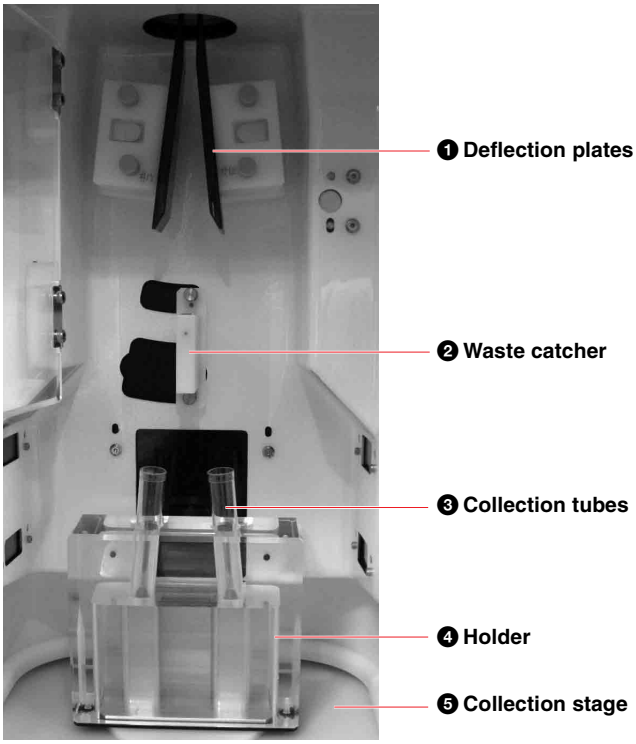
Comprises an array of dichroic beam splitter longpass filters (LPF) to separate the collected fluorescent light into separate channels, and bandpass optical filters (BPF) to filter the light entering each detector. There are eight detectors: forward scatter (FSC), back scatter (BSC), and six fluorescent light (FL1 to FL6) channels for multi-color analysis. The optical filters can be changed to tailor the detection channels for specific measurements.

Notes

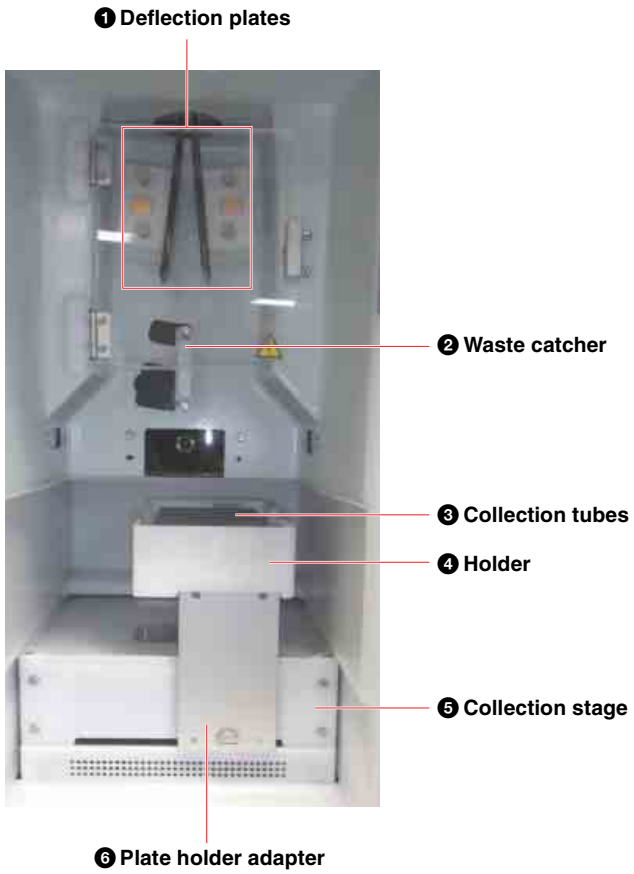
- The detection module uses components that are susceptible to static electricity, which may cause an error in operation. Do not touch the detection module during operation to prevent the transfer of static electricity.
- If you sense a discharge of static electricity from your body near the optical filters during data acquisition, it is recommended that acquisition be paused and then resumed. This procedure restores normal gain settings, even in the event that static electricity affects the operation of the detection module.

1 Sorting area

SH800 and SH800Z models



SH800ZP model



DANGER

Fluids may contain biological, chemical, or other agents. When handling collection tubes, always wear laboratory-use gloves, mask, protective goggles, and other protective clothing.

❶ Deflection plates

Deflects electrostatically-charged droplets ejected from the nozzle of the sorting chip, sorting the droplets into the designated collection tubes.
Droplets that contain target cells for sorting are charged as they are ejected from the sorting chip nozzle, while other droplets containing non-target cells are uncharged and collected in the waste catcher. The target cells for sorting are specified using gates in SH800 Software.

The trajectories of droplets that are sorted in the collection tubes are called side streams. For details, see "Side stream monitor" (page 136).

DANGER

The deflection plates are charged at an extremely high voltage. Contact with charged deflection plates can result in serious injury or electrical shock. Do not touch the deflection plates while power is connected to the main unit.

Note

Side stream calibration and data acquisition can be adversely affected by bright light shining directly on the side stream monitor. Do not place the front of the instrument near sources of bright light, such as lamps or unshaded windows.

❷ Waste catcher

Collects the uncharged, non-target droplets.
The collected droplets are passed to the waste tank in the fluidics cart.

❸ Collection tubes

Collects the electrostatically-charged, target droplets.
The SH800 supports the following collection tube devices. Each type of collection tube is used with a corresponding holder that mounts on the collection stage.

Collection tubes/ multi-well plates	Holder
15 ml conical tubes	Collection tube holder (15 ml)
5 ml round tubes	Collection tube holder (5 ml)
8-well strips	8-well strips holder[1)]
Slide glass	Slide glass holder[1)]
6-well plates	96-well plate holder
12-well plates	
24-well plates	
48-well plates	
96-well plates	
8-well PCR strips	96-well PCR plate holder
96-well PCR plates	

Collection tubes/ multi-well plates	Holder
384-well plates	384-well PCR plates[1)]
384-well PCR plates	384-well plate holder[1)] + 384-well PCR adapter[1)]

1) Options.

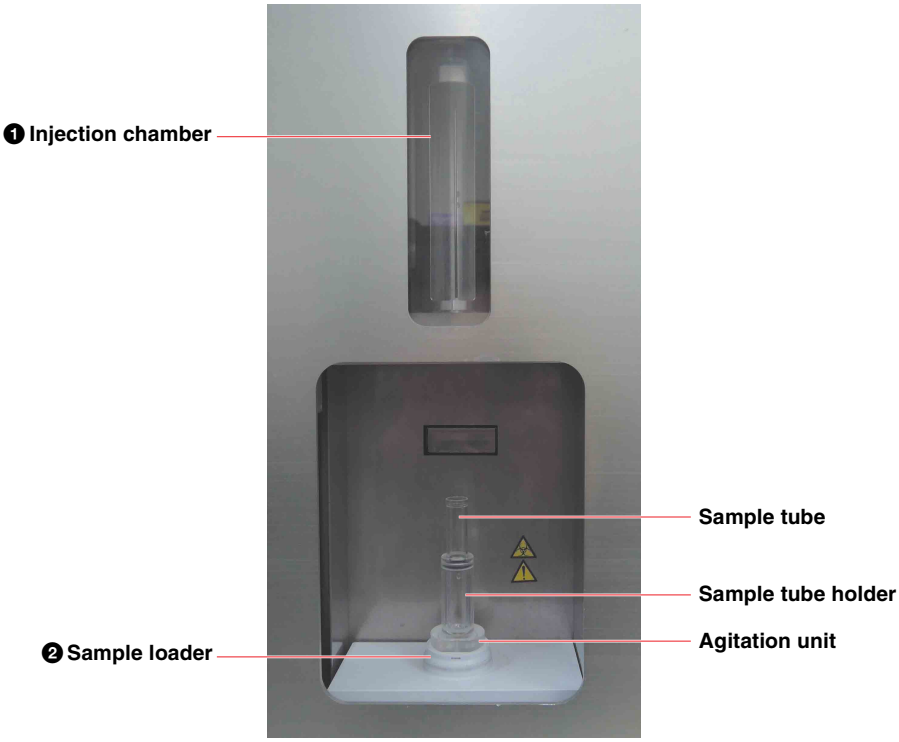
For details about loading tubes and the types of collection tubes, see "Preparing Collection Tubes" (page 33).

❹ Holder
Holds the collection tube device.

❺ Collection stage
Loads the collection tube device holder.

❻ Plate holder adapter (multi-well plates)
Used for loading multi-well plate collection devices. When running 2-way tube sorting, remove the adapter and load the corresponding collection tube holder.

2 Sample loader area



Notes

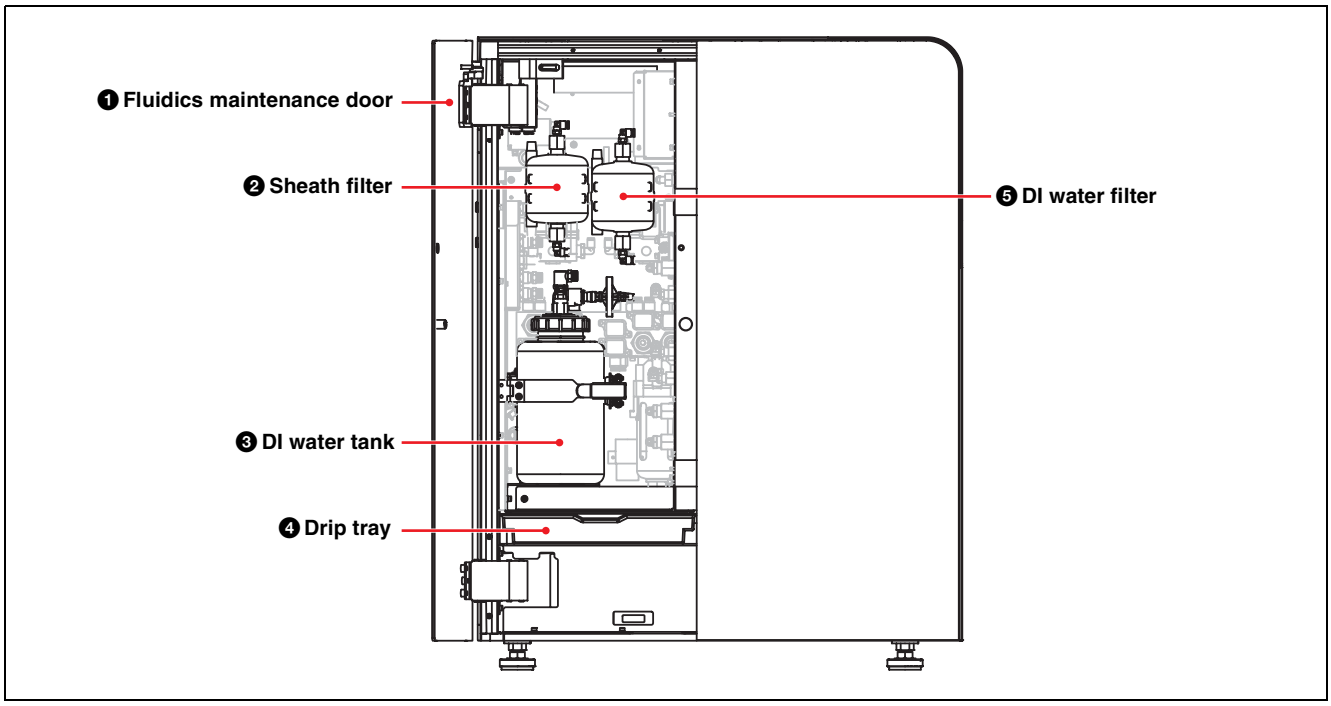
- Always wear laboratory-use gloves, mask, protective goggles, and other protective clothing when handling sample fluid to provide protection against biohazards.
- Clean the fluidics system regularly using a sodium hypochlorite liquid solution (bleach) to prevent clogging of the sample line, sample probe, and suction pump.
- Load the sample tube with its cap removed.

❶ Injection chamber
Extracts sample fluid from the sample tube, through the sample probe, and forces the fluid into the sorting chip.

❷ Sample loader
Loads a sample tube holder containing a single sample tube containing sample fluid into the sample loader agitation unit.
Samples can be loaded in 0.5 ml or 1.5 ml micro tubes, 5 ml round tubes, or 15 ml conical tubes. A sample tube holder is provided for each size of sample tube.

For details, see "Loading a Sample Tube in a Sample Tube Holder" (page 31).

Internal View (Side)



❶ Fluidics maintenance door
Provides access to the fluidics system for performing maintenance.

CAUTION
Be aware of the door edges when the door is open.

❷ Sheath filter
Filters the sheath fluid flowing between the sheath tank and the sorting chip. The sheath filter is a maintenance item.

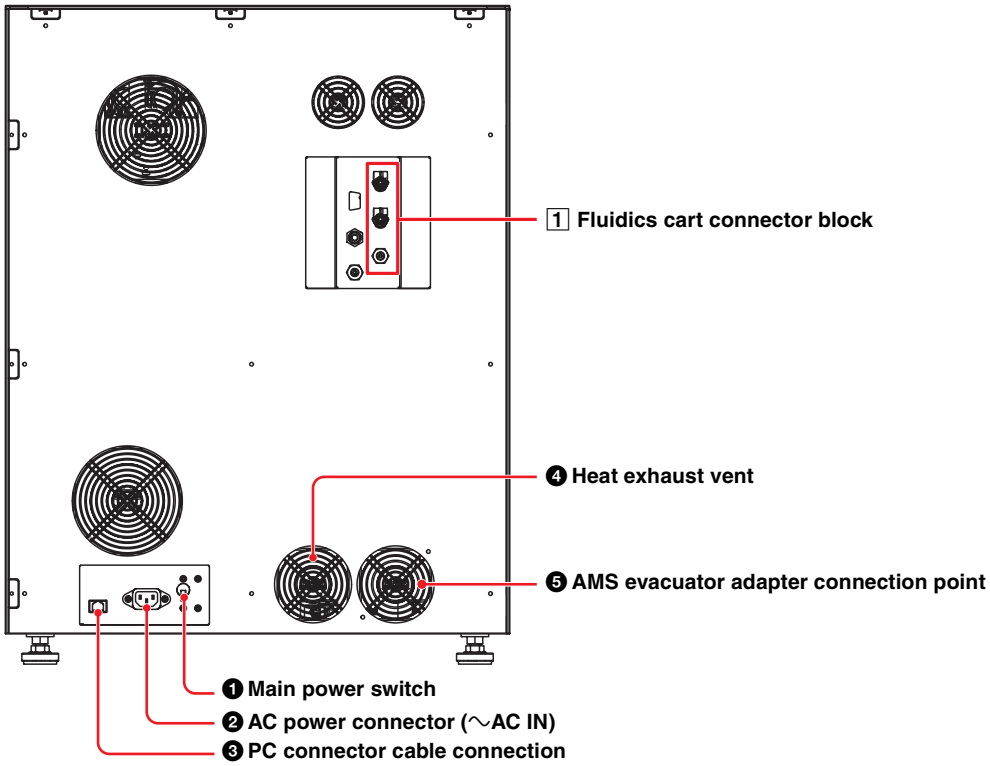
❸ DI water tank
Supplies deionized (DI) water used for flushing the fluidics system.
A sensor detects the level of DI water within the tank, and a message is displayed when the level drops below the minimum threshold.
The tank has a capacity of 1 liter (0.3 gallons US).
The DI water tank is a maintenance item.

❹ Drip tray
Collects liquid spills, during routine maintenance, and leakages due to damaged tubing or improperly fitted connectors. The drip tray should be checked regularly for signs of leakage.

An alert is shown on the host computer if fluid is detected in the drip tray. If fluid is detected, resolve the source of the leak, clean the drip tray, and wipe clean all affected surfaces. The system must be restarted after a certain time has elapsed after the alert is raised.

❺ DI water filter
Filters the DI water flowing from the DI water tank to the sample probe and other components. The DI water filter is a maintenance item.

Rear panel


1 Fluidics cart connector block
❹ Heat exhaust vent
❺ AMS evacuator adapter connection point
❶ Main power switch
❷ AC power connector (∿AC IN)
❸ PC connector cable connection

❶ Main power switch (MAIN POWER)
Turns the power supply for the SH800 ON (up) and OFF (down). The breaker automatically disconnects the power supply if an electrical fault is detected.

❷ AC power connector (∿AC IN)
Connects to a standard AC power supply (100 V to 240 V AC, 50/60 Hz). Use only the supplied power cord to connect the SH800 main unit to a power supply.

Notes

- Use only within the specified supply voltage range.
- The power cord must be connected to a power outlet with an Earth connection.

❸ PC connection cable connector (PC CONNECTION)
Connects directly to the host computer using the supplied PC connection cable.

Notes

- Do not disconnect the PC connection cable during operation. Data acquisition cannot be performed if the cable is disconnected.
- Do not connect any device other than the host computer using the PC connection cable. Device operation is not guaranteed if an external device is connected.

CAUTION

For safety, do not connect the connector to any peripheral device wiring that might apply a high voltage to this port. Follow the instructions for this port.

❹ Heat exhaust vent
Discharges heat generated from inside the unit.

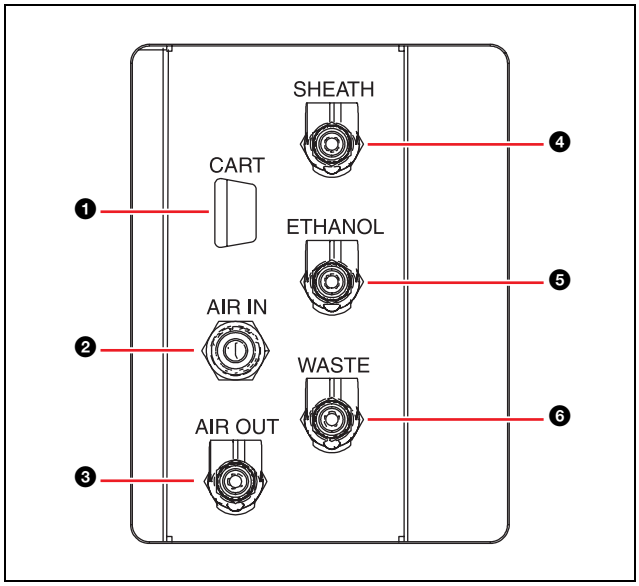
❺ AMS evacuator adapter connection point
Use a specialized adapter to connect a S.A.F.E System (evacuator) from Edge Systems LLC.

For details, see "Connecting an AMS Evacuator (SH800Z and SH800ZP)" (page 213).

Note

Maintain at least 10 cm (4 in.) space behind the main unit to allow for the dissipation of heat and to provide access to the power connector, main power switch, electrical connectors, fluidics cart connectors, and compressed air supply connectors.

1 Compressed air supply/fluidics cart connector block



1 Cart connection cable connector (CART)
Connects to the cart connection cable connector on the fluidics cart. It is used to connect the sensors for the three fluid tanks in the fluidics cart.

Note

Use only the supplied cable to connect the main unit to the fluidics cart. Using third-party cables may cause incorrect operation or device failure.

2 Compressed air supply input connector (AIR IN)
Connects to the laboratory compressed air supply or a standalone air compressor.

3 Fluidics cart air line connector (AIR OUT)
Connects to the sheath air line connector on the fluidics cart (clear tubing). The air line supplies regulated compressed air for the sheath tank.

4 Fluidics cart sheath line (blue) connector
Connects to the SHEATH connector on the fluidics cart using the supplied sheath line (blue tubing).

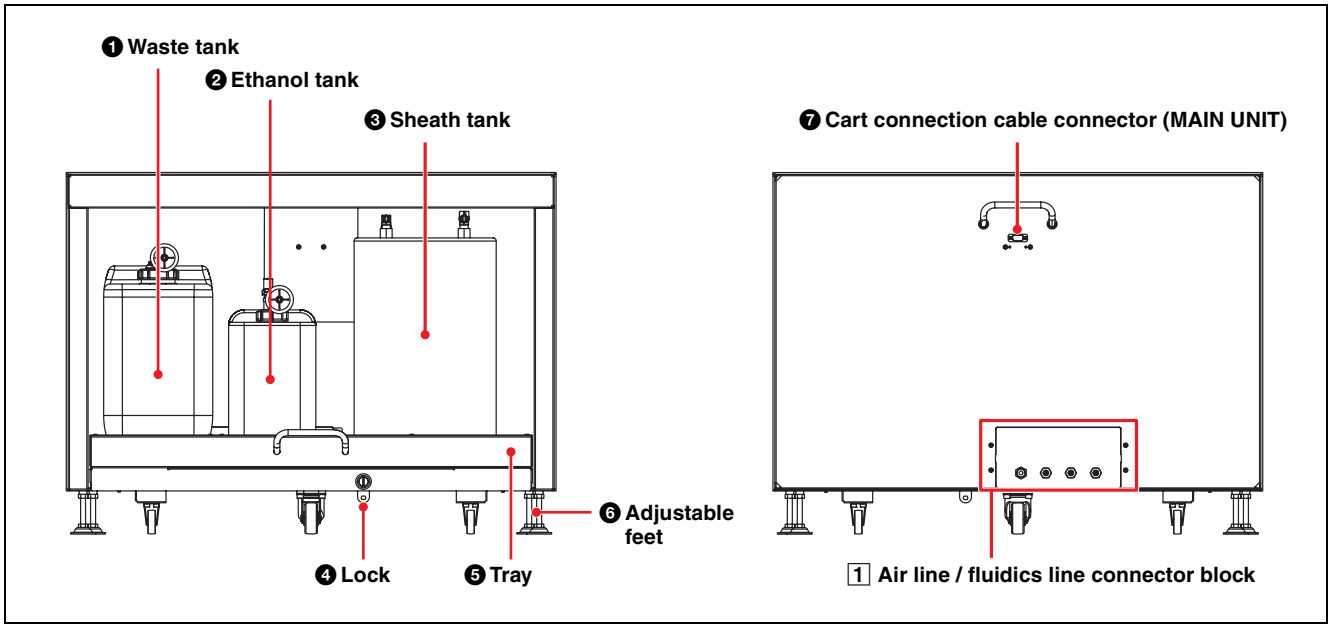
5 Fluidics cart ethanol line (green) connector
Connects to the ETHANOL connector on the fluidics cart using the supplied ethanol line (green tubing).

6 Fluidics cart waste fluid line (red) connector
Connects to the WASTE connector on the fluidics cart using the supplied waste line (red tubing).

Note

The standard fluidics lines, sheath air line, and connection cable are about 2 m (6 ft) in length. It is recommended that the SH800 be placed on a table of height approximately 700 mm (28 in.) or greater, and that the fluidics cart be placed on the floor.

Fluidics cart



1 Waste tank
Collects the waste fluid, comprising the sample fluid and sheath fluid, pumped from various points within the main unit. It also collects ethanol and DI water during cleaning. A sensor detects the waste fluid level for display on the LCD monitor and host computer. An alarm is generated and SH800 operation automatically stops when the waste fluid rises above a set level. The tank has a capacity of 10 liters (2.6 gallons US).

DANGER

Waste fluid may contain biological, chemical, or other agents. Always wear gloves and other protective clothing, mask, and goggles, as required, when emptying or replacing the waste tank. The handling of waste fluid should be performed in accordance with biological hazard handling safety procedures.

❷ Ethanol tank

Supplies the cleaning fluid (ethanol) used to clean the fluidics system during cleaning cycles. A sensor detects the ethanol level for display on the LCD monitor and host computer. An alarm is generated when the ethanol falls below a set level. The tank has a capacity of 5 liters (1.3 gallons US).

❸ Sheath tank

Supplies the sheath fluid used as the flow medium for transporting the sample fluid in a laminar flow through the sorting chip and into the collection tubes. The tank is pressurized using regulated compressed air supplied from the main unit.
A cart sensor detects the sheath fluid level for display on the LCD monitor and host computer. An alarm is generated when the sheath fluid falls below a set level. The tank has a capacity of 10 liters (2.6 gallons US).

Notes

- Never use the supplied sheath tank for anything other than the intended purpose.
- When opening the sheath tank cap, check that "Standby" is displayed on the LCD monitor or that the power to the main unit is turned off. If "Standby" is not displayed, place the unit in Standby mode using the following procedure.
  ① Click [Settings] on the [Cytometer] tab of the ribbon.
  The [Cytometer Settings] dialog appears.
  ② Click [Advanced Settings].
  The [Advanced Settings] dialog appears.
  ③ Click [Standby] on the [Pressure Options] tab.
  ④ Check that "Standby" is displayed on the LCD monitor.
  When "Standby" is displayed, disconnect the fluidics cart air line (clear tubing) from the top of the sheath tank. For details, see *"Refilling the Sheath Tank" (page 146)*.
- Do not force the lid down or place, place objects on the lid, or move the tank while the tank is pressurized.

Note

The sheath tank and sheath fluid line must not be disturbed to ensure stable, measurement conditions. Position the sheath fluid line where it will not be affected by drafts from air conditioning or vibration of the air compressor.

❺ Lock

Locks the tray.
The lock prevents the tray from being withdrawn.
To lock the tray, use a coin or similar object and turn to the LOCK position. To unlock, turn to the UNLOCK position.

❻ Tray

Holds the sheath tank, DI water tank, and waste tank, and can be withdrawn when performing maintenance.
The tray is fitted with a lock to prevent it from being withdrawn accidentally.

See "Maintenance" (page 143).

❼ Adjustable feet

Feet are adjustable to ensure the fluidics cart does not move during data acquisition.

❽ Cart connection cable connector (MAIN UNIT)

Connects to the cart connection cable connector on the main unit. It is used to connect the sensors for the three fluid tanks in the fluidics cart.

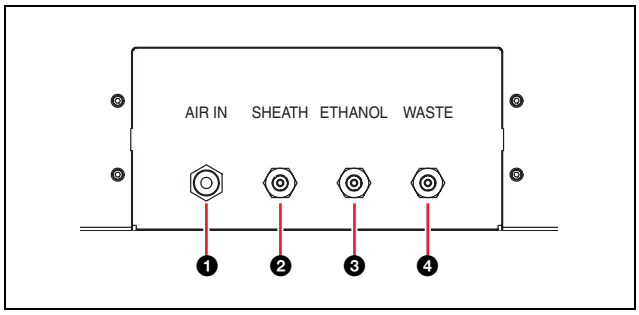
CAUTION

Use only the supplied cable to connect the main unit to the fluidics cart. Using third-party cables may cause incorrect operation or device failure.

Note

The standard cable is about 2 m (6 ft) in length.

1 Air line / fluidics line connector block



❶ Fluidics cart air line connector (AIR IN)

Connects to the fluidics cart air line connector (AIR OUT) on the SH800 (clear tubing). The air line supplies regulated compressed air for the sheath tank.
The SH800 is designed to connect to 500 kPa to 650 kPa (73 psi to 94 psi) compressed air supply for stable output.

❷ Fluidics cart sheath line (blue) connector

Connects to the SHEATH connector on the main unit using the supplied sheath line (blue tubing).

❸ Fluidics cart ethanol line (green) connector

Connects to the ETHANOL connector on the main unit using the supplied ethanol line (green tubing).

❹ Fluidics cart waste fluid line (red) connector

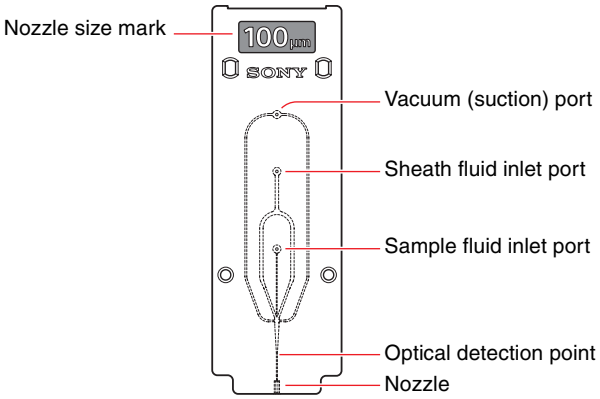
Connects to the WASTE connector on the main unit using the supplied waste line (red tubing).

Notes

- The standard fluidics lines and cable are about 2 m (6 ft) in length. Use of lines longer than 2 m (6 ft) may impair the pumping of fluids from the fluidics cart.
- Use only the supplied cable to connect the main unit to the fluidics cart. Using third-party cables may cause incorrect operation or device failure.

Sorting Chip

The SH800 uses a disposable sorting chip.



Sample fluid and sheath fluid enter the chip through inlet ports on the front surface of the chip. The sample fluid and sheath fluid are forced into the chip by air pressure from the sample loader and the sheath tank, respectively. The sample fluid and accompanying sheath fluid flows through the chip where the sample fluid is focused into a stream of single-file cells. The cells in the sample fluid then pass the optical detection point where they are interrogated by the lasers, before being ejected from the nozzle. A transducer vibrates the chip at an ultrasonic frequency to break the fluid stream into droplets for sorting.
A vacuum port provides negative pressure (suction) to remove bubbles and clogged samples from the fluidics channels in the sorting chip.
The nozzle size mark indicates the size of the nozzle located at the bottom of the chip.

The sorting methods that can be used vary depending on the nozzle diameter of the sorting chip and the size of cells. For details, see "Sorting" (page 53).

Note

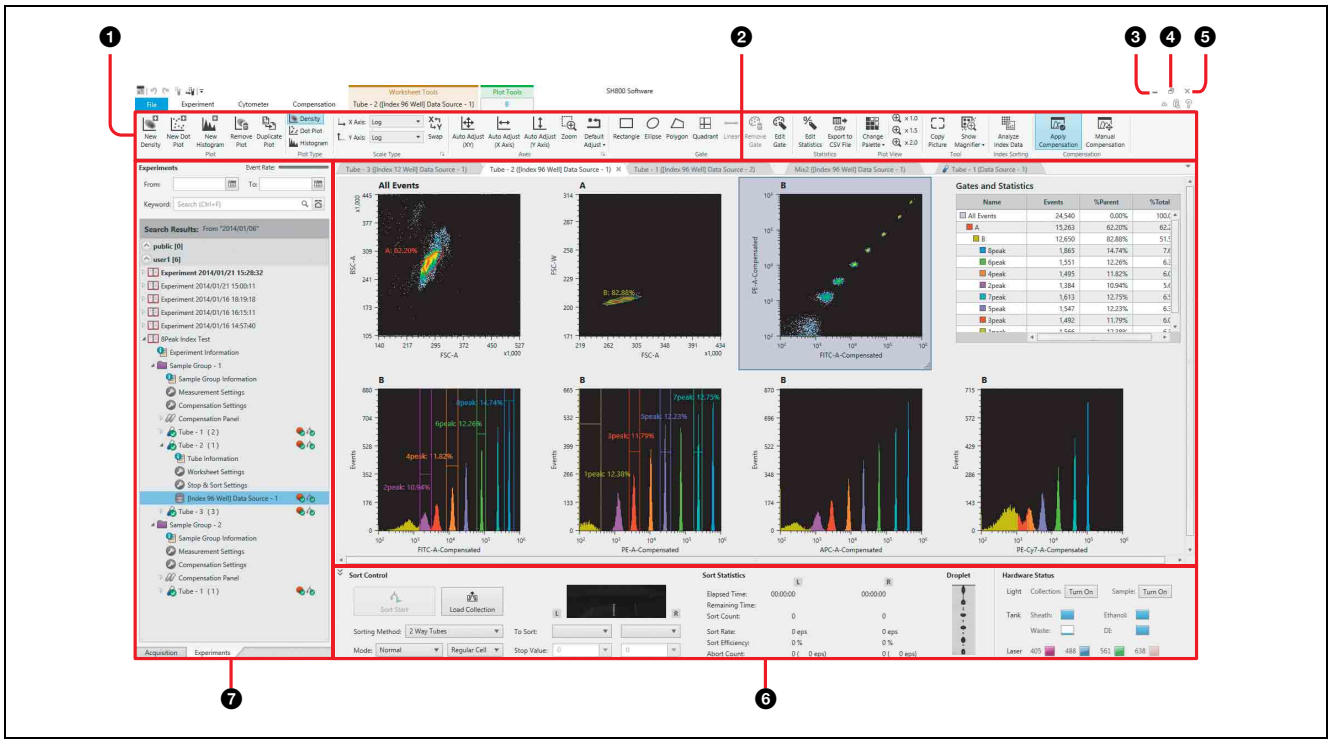
Do not touch the sorting chip near the optical detection point. Fingerprints and smudges near the optical detection point can adversely affect data acquisition results.

Main Window

Notes

- The menus and buttons displayed vary depending on the user account privileges.
- The screenshots displayed in this document may vary from the actual software.

Operating window



For details about the items displayed, see "Chapter 7 Window Description" (page 104).

❶ Ribbon
Displays the tool buttons that can be used in the displayed window.
The content of the ribbon varies depending on the selected tab.

❷ Worksheet
Work area for displaying and editing the results from data acquisition and analysis.

❸ Ribbon show/hide control (minimize/restore)
Clicking / alternately shows the ribbon or hides the ribbon.

❹ Logout
Logs out the currently logged in user and displays the login window *(page 42)*.

❺ Help
Displays the Help window for accessing the Operator's Guide and to check software version information.

❻ Sorting control pane
Configures and controls droplet sorting.
It also displays the current status of sorting, fluid tank levels, lights, and laser sources.
The displayed items vary depending on the model and the sorting method.
The sorting control pane is a collapsible window element. The panel can be shown or hidden as required.

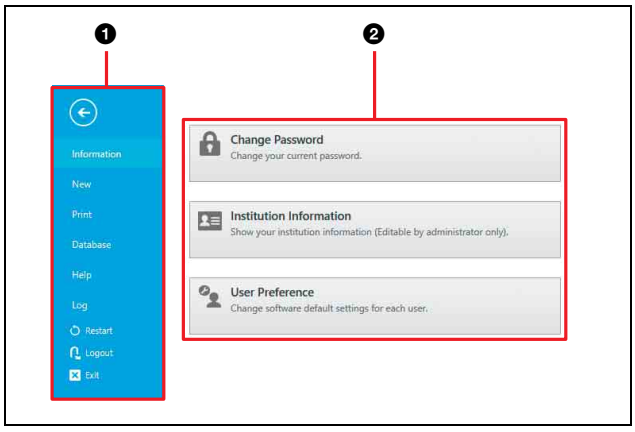
Tip

When the unit is calibrated in analyzer mode, the sorting control pane is automatically hidden.

❼ Experiment/Acquisition control area
Used to configure and control data acquisition and work with experiments.
[Acquisition] tab: Displays the controls used for acquiring and recording data.
[Experiments] tab: Displays experiments and the components in an experiment, used to acquire and analyze data, in a hierarchical list view.

[File] window

Example: [Information] tab



❶ Menu items
Displays the following menu items.
[Information]: Displays commands used to configure user passwords and institution information, and to add/manage user accounts.
[New]: Creates a new experiment based on saved templates and/or recent experiments.

[Print]: Prints analysis results and other reports.
[Database]: Displays commands used to perform experiment database operations and to check hard disk free capacity.
[Help]: Displays commands used to display the Operator's Guide and to check software version information.
[Log]: Displays commands used to display the chip alignment log, sorting calibration log, and user log.
[Restart]: Restarts SH800 Software.
When the software restarts, it loads the same conditions that existed at the time [Restart] was pressed, but without assigning any experiment as the active experiment.

Tip

The restart function should only be used when the SH800 Software is running very slowly or is otherwise unstable. It should not be used during normal operation.

[Logout]: Logs out the currently logged in user and displays the login window.
[Exit]: Exits SH800 Software.

❷ Operation area
Displays function buttons and information related to the menu item selected on the left side.

SH800 Software Operating Environment

The host computer must satisfy the following requirements in order to run SH800 Software.

Operating system (OS): Microsoft Windows 8 Operating System, Professional, 64 bit
CPU: Intel Core i7-3820QM 2.70 GHz or later
Cache: Level 3 cache memory 8 MB or greater
Memory: PC3-12800, 1600 MHz, 16 GB (8 GB × 2) or greater
Network interface: 1000BASE-T (1)
Main storage: 1 TB HDD (SATA 1 TB × 1) or greater
Monitor resolution: 1920 × 1080 (16:9 Full HD)
Graphics resolution: 1920 × 1080 (Full HD)
Camera: 1.3 megapixels or higher effective pixels
OS language setting: Symbol for the decimal point is "." (period).

Note

If the decimal point is set to a character other than"." (period), the following error message is displayed when starting SH800 Software.

Invalid Language setting was detected.
Please change "Decimal symbol" setting to ".(period)" according to the Operator's Guide.
Press [OK] to close this application.

If this occurs, use the following procedure to change the decimal point to "." (period), then restart SH800 Software.

1. Open [Control Panel] > [Clock, Language, and Region] > [Region and Language].
2. Open [Additional settings...] on the [Formats] tab.
3. Set [Decimal symbol] to "." (period) on the [Numbers] tab.

LCD Monitor

The LCD monitor on the front of the SH800 displays status information during operation.

The LCD monitor is turned on/off using the Display Mode button on the front panel.

Tip

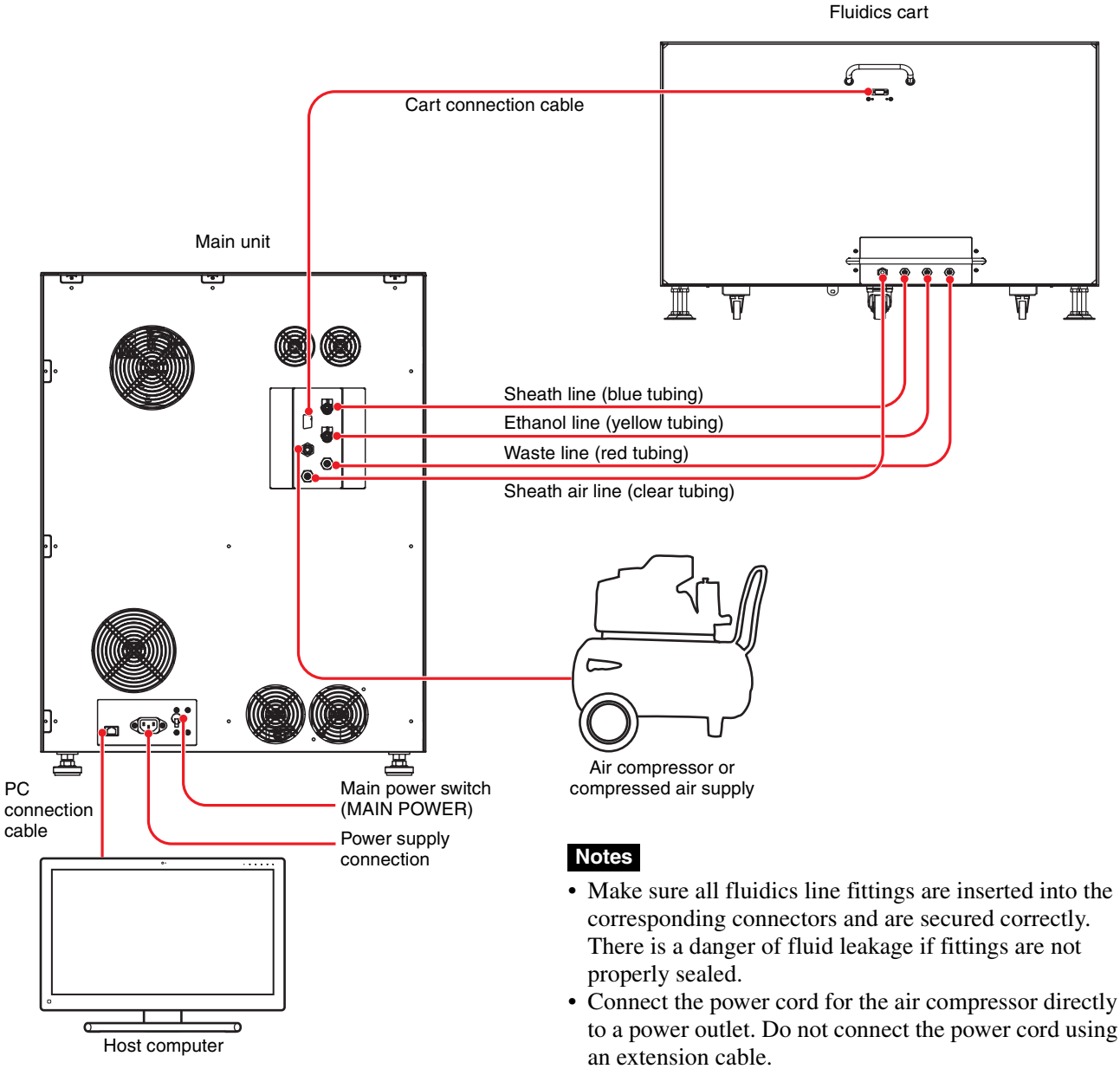
If conflicting information is displayed in SH800 Software display and on the LCD monitor, it does not necessarily indicate a failure or error.

Preparation

Chapter 2

Checking Device Connections

Check that the main unit, fluidics cart, host computer, and compressed air supply are connected correctly.

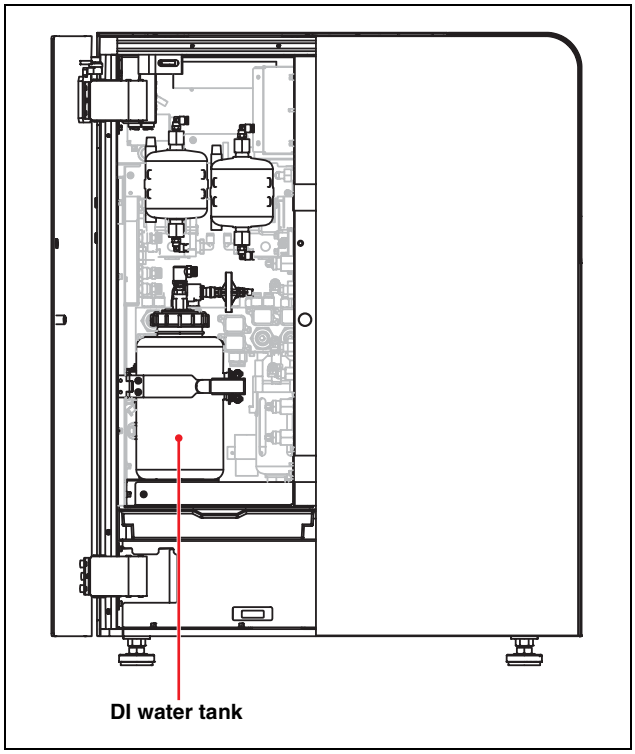


Notes

- Make sure all fluidics line fittings are inserted into the corresponding connectors and are secured correctly. There is a danger of fluid leakage if fittings are not properly sealed.
- Connect the power cord for the air compressor directly to a power outlet. Do not connect the power cord using an extension cable.

Filling the DI Water Tank

Fill the DI water tank in the main unit with DI water.
DI water is used to flush sheath fluid, ethanol, and other cleaning agents from the fluidics system.
Always check the level in the DI water tank and refill as required before starting/shutting down the SH800 and before running ethanol cleaning.



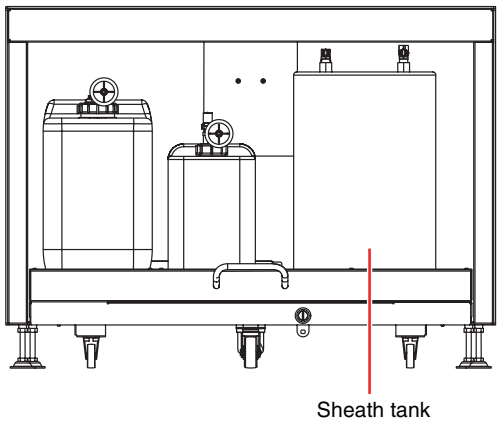
For details about refilling the DI water tank on the fluidics cart, see "Refilling the DI Water Tank" (page 149).

Tip

An alarm is raised if the level in the DI water tank drops too low.

Filling the Sheath Fluid Tank

Fill the sheath fluid tank on the fluidics cart with sheath fluid.
Sheath fluid is used as a transport media to deliver sample fluid to the sorting chip, and to clean the sample line.
Before starting and shutting down the SH800, make sure that there is sufficient sheath fluid in the sheath tank, and refill as required.



For details about refilling the sheath tank on the fluidics cart, see "Refilling the Sheath Tank" (page 146).

Note

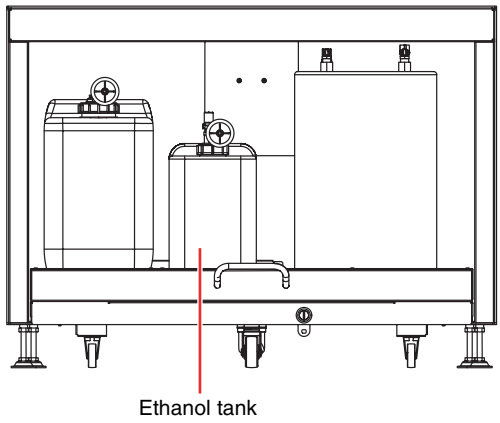
Always place the sheath tank in the center of the tray area. The fluid level detector may not operate correctly if the tank is placed off-center.

Tip

An alarm is raised if the level in the sheath tank drops too low.

Filling the Ethanol Tank

Fill the ethanol tank on the fluidics cart with ethanol. Ethanol is used during ethanol cleaning to disinfect the fluidics system.
Always check the level in the sheath tank and refill as required before running ethanol cleaning.



For details about refilling the ethanol tank on the fluidics cart, see "Cleaning the Internal Sheath Line and DI Water Line using Ethanol" (page 188).

Note

Always place the ethanol tank in the center of the tray area. The fluid level detector may not operate correctly if the tank is placed off-center.

Tip

An alarm is raised if the level in the ethanol tank drops too low.

Preparing a Sample

Loading a Sample Tube in a Sample Tube Holder

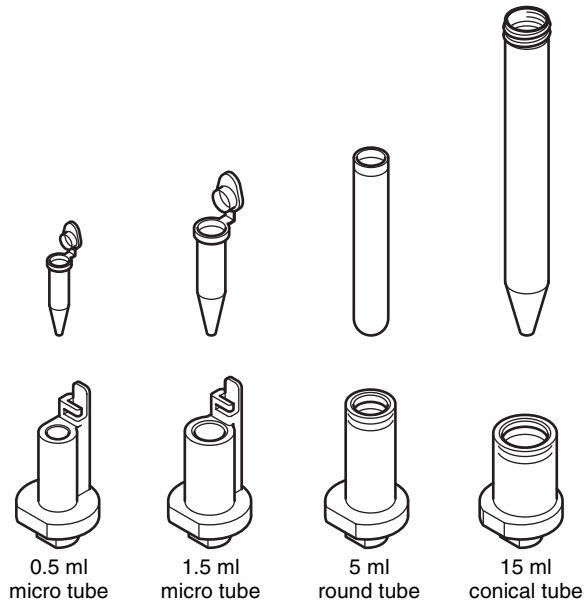
Insert the sample fluid for analysis or sorting in a sample tube, and place the tube in the corresponding sample tube holder.
The SH800 supports the following sample tubes.
Each sample tube is used with a corresponding holder that mounts in the sample loader.

Sample tube	Holder
0.5 ml micro tubes	Sample tube holder (0.5 ml)
1.5 ml micro tubes	Sample tube holder (1.5 ml)
5 ml round tubes	Sample tube holder (5 ml)
15 ml conical tubes	Sample tube holder (15 ml)

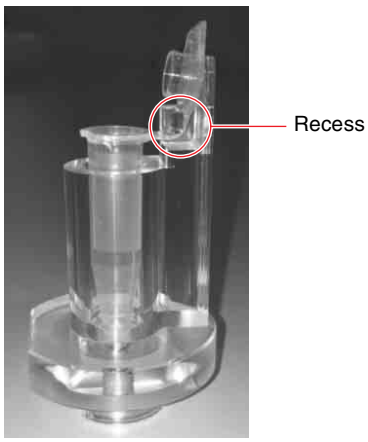
Load the sample tube into the corresponding size sample tube holder.

Note

Always remove the sample tube caps when performing measurements.



The tube holders for micro tubes have a recess at the top to hold the cap clear of the tube opening.



Notes

- Always wear laboratory-use gloves, mask, protective goggles, and other protective clothing when handling sample fluid to provide protection against biohazards. Also, take care when loading the sample tube in the tube holder to avoid sample fluid spills and breakage of the sample tube.
- Before using a tube, check that there is no dust or other foreign matter in the tube.

Preparing Automatic Setup Beads

Prepare automatic setup beads in advance if planning to run automatic calibration.

1 Filter the automatic setup beads using a 30 µm filter.

2 Insert 1 ml or more of undiluted, automatic setup beads in a sample tube, and place the tube in the corresponding sample tube holder.

Always use automatic setup beads in undiluted form.

Sample Temperature Control

The SH800 is fitted with thermal control units in the sample loader and collection stage to maintain sample fluid temperature control while running an experiment. The temperature control device provides basic control of temperature and can be set to 5 °C (41 °F) or 37 °C (98.6 °F). You configure settings in SH800 Software.

Notes

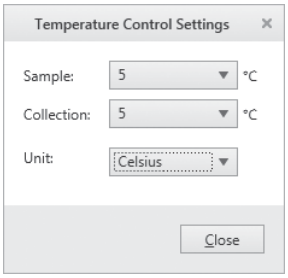
- The temperature control function in SH800 Software cannot be used to cool 8-well strip holders/slide glass holders, 96-well plate holders, 384-well plate holders, or 384-well PCR adapters. However, the holders contain an embedded cooling agent. You can cool a holder in a laboratory refrigerator (–20 °C (–4 °F)) beforehand, and then place the holder on the collection stage.
  – For 8-well strip holders/slide glass holders and 96-well plate holders:
    Cooling time: 5 hours or longer
    Temperature retention time: About 1 hour (varies with ambient temperature)
  – For 384-well plate holders and 384-well PCR adapters:
    Cooling time: 8 hours or longer
    Temperature retention time: About 1 hour (varies with ambient temperature)
- Note that holders/adapters may freeze if immediately placed on the collection stage when removed from the refrigerator.

For details about starting the SH8000 instrument and SH800 Software, see "System Startup" (page 41) and "Logging In" (page 42).

1 Click the dialog launcher in the [Temperature Control] group on the [Cytometer] tab of the ribbon.

The [Temperature Control Settings] dialog appears.

2 Select the temperature setting for the sample fluid, then click [Close].



Sample: 5 °C (41 °F) or 37 °C (98.6 °F)
Collection: 5 °C (41 °F) only

Note

When sorting with the temperature set to 5 °C (41 °F), place the collection tube holder in a refrigerator to cool the holder before loading it on the collection stage.

3 To turn a temperature control device on/off, click [Sample] or [Collection] in the [Temperature Control] group on the [Cytometer] tab of the ribbon.

The buttons toggle between on and off.

Note

If the temperature control function is turned off, expected acquisition results may not be obtained, depending on the sample being analyzed.

Precautions when temperature control is set to 5 °C (41 °F)

- Cool the collection holder to approximately 5 °C (41 °F) in a refrigerator before loading it onto the collection stage. Cooling the collection holder beforehand significantly reduces the time required to cool the collection tubes.

- While the temperature is set to 5 °C (41 °F), do not remove the collection tube holder from the collection stage. Insert and remove the collection tubes directly from the collection tube holder.
- Loading a collection tube holder that is warm or that has become warm may result in a sudden change in temperature, perhaps initiating a temperature control error causing the main unit to be reset. When unloading and loading the collection tube holder, temporarily turn off the temperature control function, wait about 30 seconds, and then set the temperature to 5 °C (41 °F) again.
- The metallic parts of the 96-well plate holder become cold when the holder is cooled. Always load and unload by holding the plastic sides.

Preparing Collection Tubes

Prepare the collection tube device for collecting droplets containing target cells for sorting.

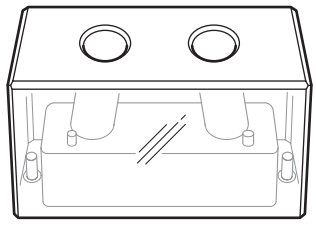
The SH800 supports the following collection tube devices. Each type of collection tube is used with a corresponding holder that mounts on the collection stage.

Collection tubes/ multi-well plates	Holder
15 ml conical tubes	Collection tube holder (15 ml)
5 ml round tubes	Collection tube holder (5 ml)
8-well strips	8-well strips holder[1)]
Slide glass	Slide glass holder[1)]
6-well plates	96-well plate holder
12-well plates	
24-well plates	
48-well plates	
96-well plates	
8-well PCR strips	96-well PCR plate holder
96-well PCR plates	
384-well plates	384-well plate holder[1)]
384-well PCR plates	384-well plate holder[1)] + 384-well PCR adapter[1)]

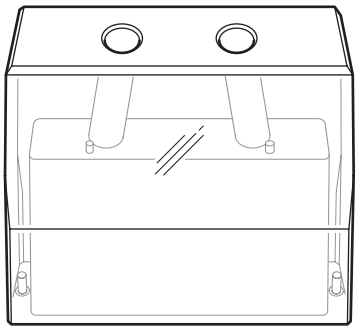
1) Options.

Common to all models

- Collection tube holder (15 ml)



- Collection tube holder (5 ml)

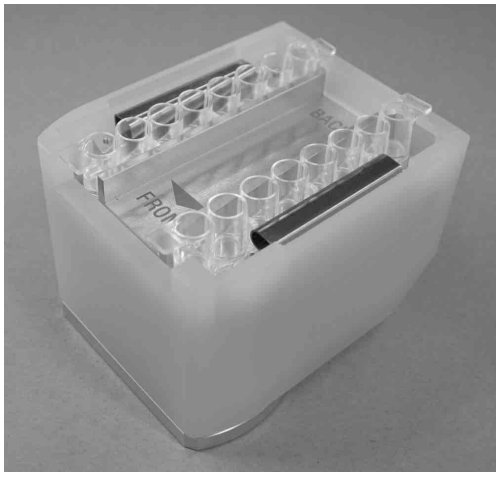


When running 2-way tube sorting on the SH800ZP, the plate holder adapter must first be removed. See

"Removing the Plate Holder Adapter (SH800ZP only)" (page 37).

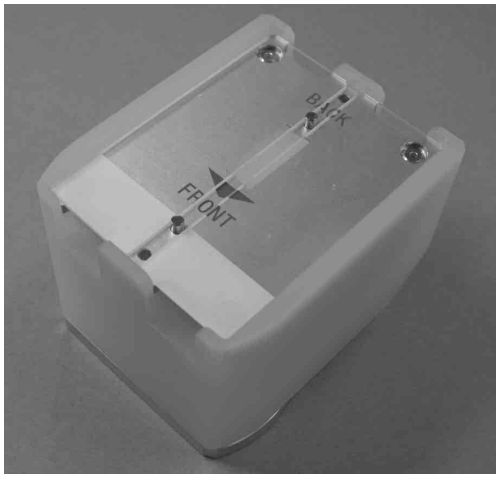
SH800 and SH800Z

- 8-well strips holder (with 8-well strips)



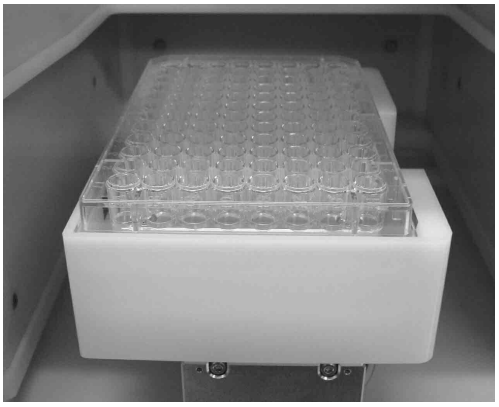
For details about loading 8-well strips, see "Loading 8-well Strips (SH800 and SH800Z)" on page 35.

- Slide glass holder

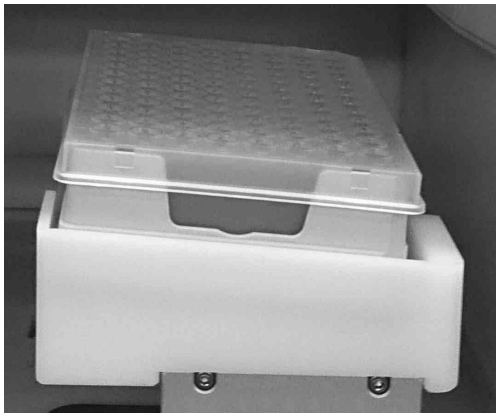


SH800ZP only

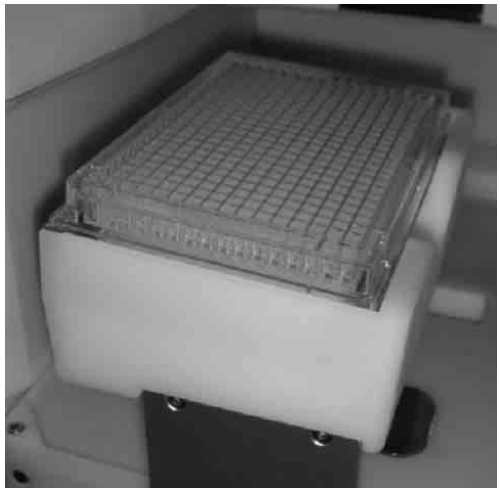
- 96-well plate holder (with 96-well plate)



- 96-well PCR plate holder (with 96-well PCR plate)



- 384-well plate holder (with 384-well plate)



For details about loading tubes, see "Loading a Multi-well Plate (SH800ZP only)" (page 36).

- 384-well plate holder (with 384-well PCR adapter and 384-well PCR plate)

For details about loading tubes, see "Loading a 384-well PCR Plate" (page 36).

When sorting into a 384-well plate or 384-well PCR plate, always adjust the sort position before starting. For details, see *"Adjusting the Sort Position (SH800ZP only)" (page 99).*

DANGER

Fluids may contain biological, chemical, or other agents. When handling collection tubes, always wear laboratory-use gloves, mask, protective goggles, and other protective clothing.

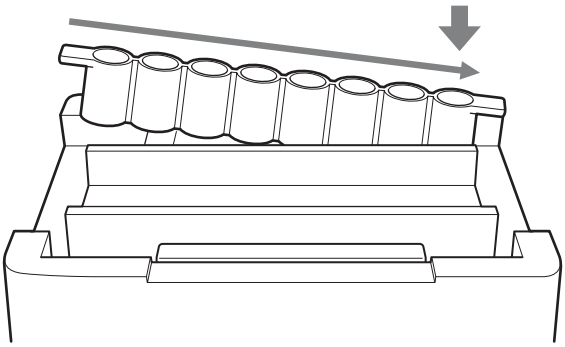
Note

Before using a tube, check that there is no dust or other foreign matter in the tube.

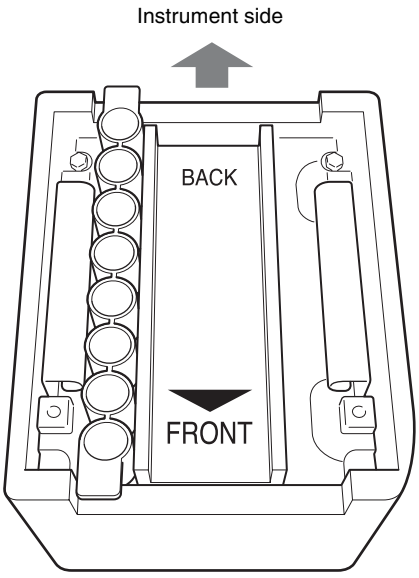
Loading 8-well Strips (SH800 and SH800Z)

Observe the following precautions when loading 8-well strips.

1 Insert the leading edge of the 8-well strip into the holder at an angle, and push the plate in all the way.

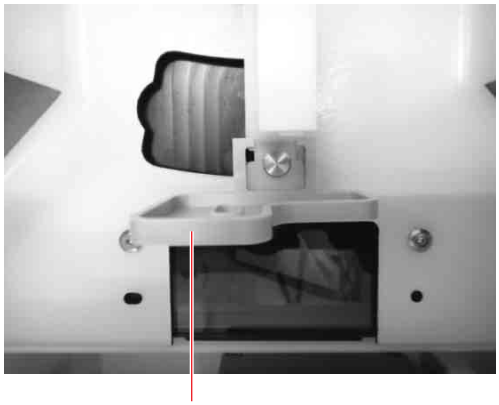


2 Orient the holder with the "FRONT" arrow towards you and place the holder on the collection stage.

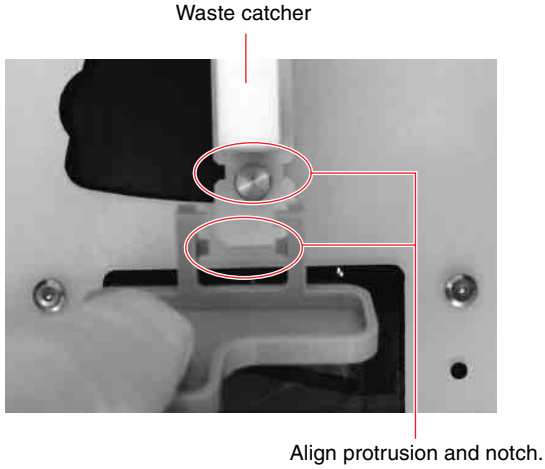


Attaching the Splash Guard (for use with multi-well plates) (SH800ZP only)

Attach the supplied splash guard when sorting into multi-well plates to prevent splashing of sample fluid.

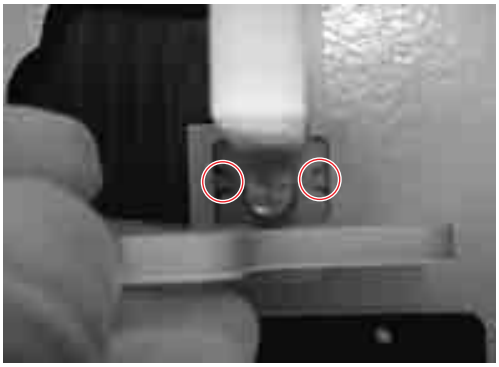


1 Hold the splash guard in the orientation shown in the diagram, and insert the protrusion on the splash guard into the notch on the waste catcher.

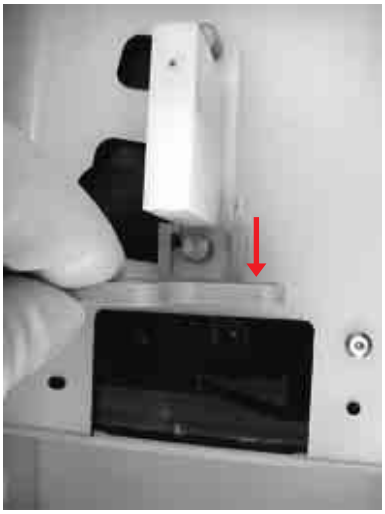


⇩

Insert the protrusions on both sides.



2 Push the splash guard in until it clicks into position.

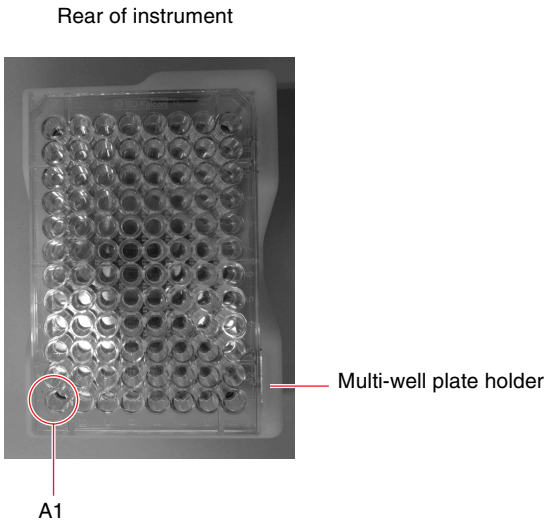


Tip

Remove the splash guard when unloading multi-well plates after sorting is finished.

Loading a Multi-well Plate (SH800ZP only)

Place the multi-well plate into the plate holder with the chamfered corners toward the rear of the instrument.

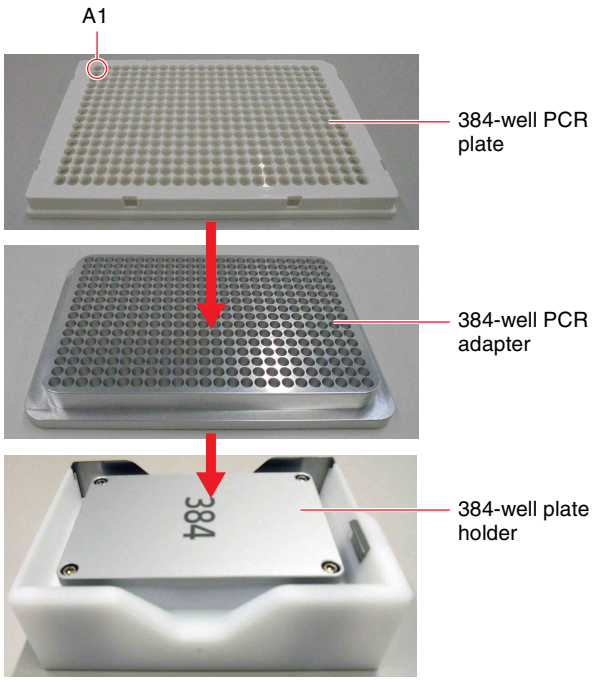


Notes

- Always use a 384-well plate holder when using 384-well plates. Do not use a 96-well plate holder.
- Always use a 96-well PCR plate holder when using 96-well PCR plates. Do not use a 384-well plate holder.

Loading a 384-well PCR Plate

Place the 384-well PCR adapter in the 384-well plate holder, and place a 384-well PCR plate on the adapter. Place the 384-well PCR plate into the adapter with the A1 well at the front left of the instrument.



Note

Always use a 384-well PCR plate holder and 384-well PCR adapter when using 384-well PCR plates. Do not use a 96-well PCR plate holder.

Removing the Plate Holder Adapter (SH800ZP only)

When running 2-way tube sorting, the plate holder adapter must first be removed.

Remove the screw securing the adapter using a coin or screwdriver, and remove the adapter.

⇩

Configuring Users

This section describes how to manage user accounts in SH800 Software.

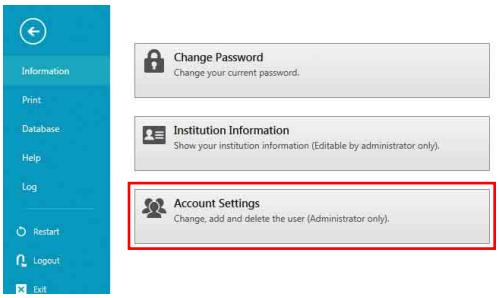
For details about starting the SH8000 instrument and SH800 Software, see "System Startup" (page 41) and "Logging In" (page 42).

Adding Users

Only administrators can add new users.

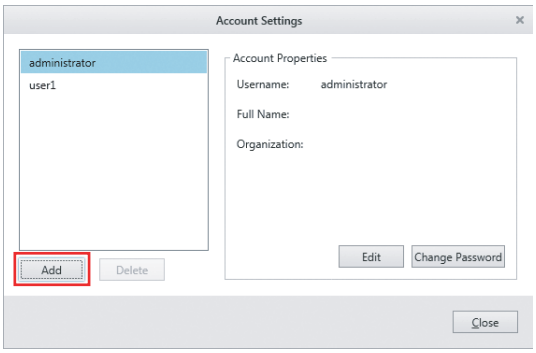
1 Click the [File] tab, then click [Information] in the menu on the left.

2 Click [Account Settings].



The [Account Settings] dialog appears.

3 Click [Add].

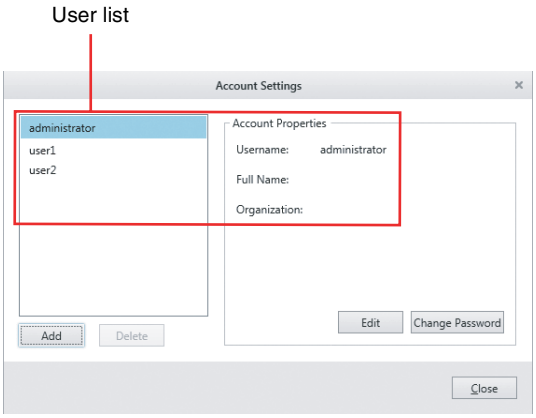


The [Add Account] dialog appears.

4 Set each field, then click [OK].

[Username]: Enter the user name, of up to 32 alphanumeric characters, used when logging in to SH800 Software (required item).
[Password]: Enter the password, comprising up to 20 alphanumeric characters, used when logging in to SH800 Software (required item).
[Confirm Password]: Enter the same password again for confirmation (required item).
[Full Name]: Enter the full name of the user.
[Organization]: Enter the name of the organization.
[Accessibility]: Place a check mark in the checkbox if you want to temporarily disable a general user account.

The user is registered in the user list in the [Account Settings] dialog.



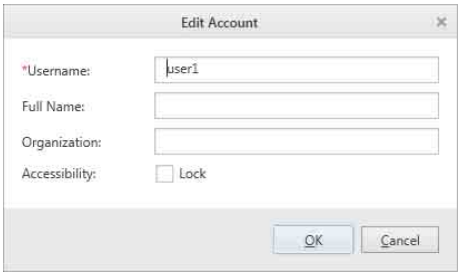
Editing User Settings

Only administrators can edit user settings.

1 Select the user you want to edit in the [Account Settings] dialog, then click [Edit].

Selecting a user in the list displays the settings for the user in the area on the right.
The [Edit Account] dialog appears.

2 Edit the settings, then click [OK].



For details, see step ***4*** *(page 38) in "Adding Users".*

The account settings are updated.

Changing Passwords

Individual users can change their password used to log in to SH800 Software.

1 Click the [File] tab, then click [Information] in the menu on the left.

2 Click [Change Password].

The [Change Password] dialog appears.

3 Set each field, then click [OK].

[Old Password]: Enter the current password (required item).
[New Password]: Enter a new password, comprising up to 20 alphanumeric characters (required item).
[Confirm Password]: Enter the same password again for confirmation (required item).

The password is updated.

Chapter 3 Basic Operation

This chapter describes the basic operating procedure for running a simple experiment, acquiring and analyzing data, and sorting selected populations in the sample.

Workflow

Step 1	**Start the system** ***(page 41)*** Turn on the power to start the SH800.
⬇	
Step 2	**Log in** ***(page 42)***
⬇	
Step 3	**Setup the instrument** ***(page 43)*** Configure the required settings for data acquisition.
⬇	
Step 4	**Run automatic calibration** ***(page 46)*** Run automatic calibration to align the sorting chip and calibrate droplet formation using automatic setup beads.
⬇	
Step 5	**Create an experiment** ***(page 47)***
⬇	
Step 6	**Acquire data** ***(page 49)***
⬇	
Step 7	**Analyze data** ***(page 51)***
⬇	
Step 8	**Sorting** ***(page 53)***
⬇	
Step 9	**Clean fluidics system** ***(page 143)*** Clean the sample probe, sample line, and sheath line.
⬇	
Step 10	**Shut down** ***(page 65)*** Shut down the main unit, and clean the sample loader and sorting area.

System Startup

Turn on the power supply and start the SH800 using the following procedure.

Note

Do not open the flip-up door at any time before turning on the compressed air supply. Doing so may damage the unit.

1 Check that the compressed air supply is connected and is supplying the rated pressure.

2 Check the level in the tanks on the fluidics cart, and refill or empty the tanks as required.

Also, check that the fluidics lines, sheath air line, and cart connection cable are connected correctly.

3 Turn on the MAIN POWER switch on the rear panel of the main unit.

Note

During normal operation, the MAIN POWER switch can be left turned on all the time. Use the POWER/STANDBY button to turn power on/off on a daily basis.

4 Turn on the power using the POWER/STANDBY button on the front panel of the main unit.

The system goes through an initialization process during which self-diagnostics are run to test the proper working condition of all subsystems.

The SH800 status appears on the LCD monitor.

Logging In

Start SH800 Software and log in to the SH800.

Note

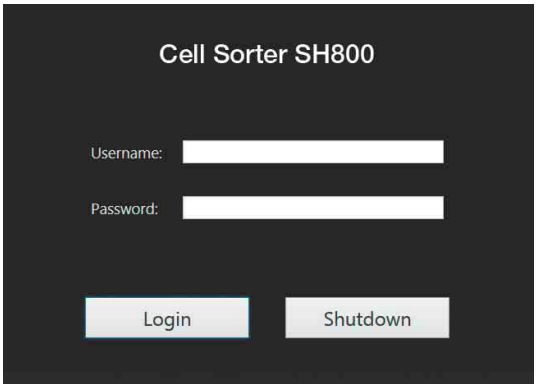
Only one user has permission to operate the SH800 at any one time. Other users may log in to monitor the operation, but they do not have permission to operate the SH800.

1. Select SH8000 Software on the Start screen to start the software.

   The login window appears.

2. Check that “Standby” status is displayed on the LCD monitor.

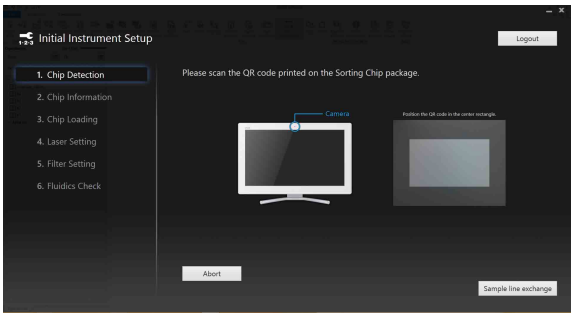
3. Enter your user name and password, then click [Login].



Tip

The administrator account user name and password are both set to “administrator” when the unit is shipped. It is recommended that you change the password for the administrator account for security considerations.

If login is successful, the [Initial Instrument Setup] wizard appears.



Tip

To change the sample line before initial instrument setup, click [Sample line exchange] and follow the instructions in the on-screen wizard.

For details, see “Changing the PEEK Sample Line (PEEK sample line compatible models)” (page 169) or “Changing the Sample Line and Probe (Non-PEEK sample line compatible models)” (page 172).

Initial Instrument Setup

Load a sorting chip, select the lasers to be used, and insert the optical filters in the required optical filter pattern by following the on-screen instructions in the [Initial Instrument Setup] wizard.

1 Hold the QR code on the sorting chip packaging in front of the camera on the host computer.

QR code

The camera automatically scans the QR code.

Tip

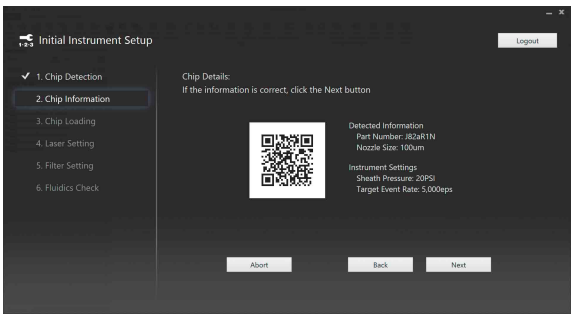
You can click [Abort] to bypass instrument configuration and proceed directly to the main window. In this mode, SH800 Software can be used only for analysis of existing recorded data in experiments.

When loading a sorting chip, the QR code on the chip packaging must be scanned using the built-in camera on the host computer to uniquely identify the chip and register the serial number and nozzle size of the chip with the SH800. This function maintains a history for the chip in order to ensure optimum performance.

When the QR code is successfully scanned, the part number and nozzle size are displayed, together with basic instrument settings. The serial number for the chip is registered internally.

When the sorting chip is scanned successfully, the serial number and other information for the chip appears.

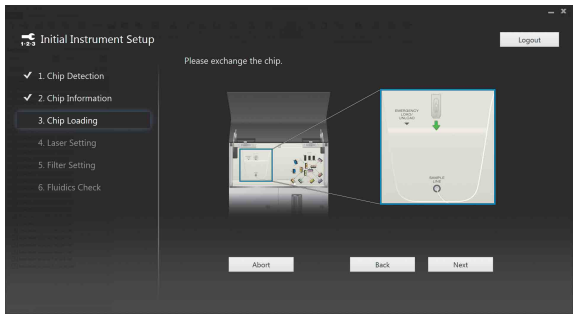
2 Check that the displayed information is correct, then click [Next].



The currently loaded sorting chip, if one is present, is ejected from the chip loader for removal.

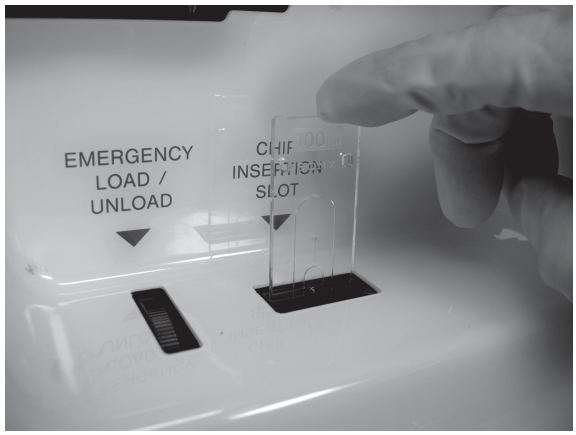
Tip

If you wish to load a different chip, click [Back] to return to the previous screen, then scan the QR code for the different chip.



3 Open the flip-up door to access the chip loader.

4 Remove the current sorting chip, if one is present, and insert the new chip into the chip slot at the top of the chip loader.



Insert the chip into the slot with the Sony logo and nozzle size mark at the top facing toward you.

Place the previous chip in its original packaging. The chip and its corresponding QR code should be kept together at all times so that they are not mixed up with other chips.

Notes

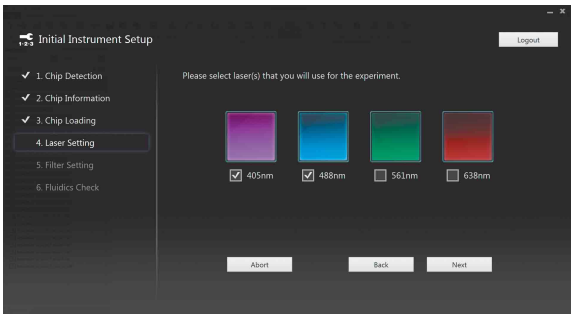
- An error message appears if the sorting chip is inserted in the incorrect orientation.
- Do not insert any objects other than Sony sorting and cleaning chips designed for the SH800 into the chip insertion slot.
- The packaging for the loaded sorting chip should be stored in a safe location.
- Replace the sorting chip if it is discolored, dirty, or stained from previous use.
- Do not drop the sorting chip on a hard surface or bend the chip intentionally. If there is a problem with the sorting chip, contact your Sony distributor.

- Always wear gloves and other protective clothing, mask, and goggles, when changing the sorting chip.
- If the sorting chip will not load correctly, run the instrument in maintenance mode as described in *"Running Maintenance Mode" (page 158)* to clean the instrument. If the problem persists, contact your Sony distributor for service.
- Replace the sorting if it is used for extended periods or after the usage period expires, and when it becomes clogged, discolored, or stained.

5 Click [Next].

The sorting chip is loaded and the [Laser Setting] screen appears.

6 Select the excitation lasers to be used for the experiment, then click [Next].



The excitation spectra of the fluorochrome labels you wish to detect largely determine the lasers needed for an experiment.

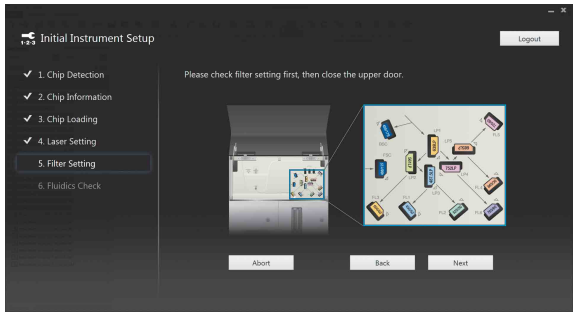
For a list of fluorochromes versus excitation laser wavelength for each model, see "Fluorochrome Detection Matrix" (page 204).

Tips

- The 488 nm excitation laser is used to measure forward scatter and back scatter light.
- It is recommended that lasers not required for an experiment be deselected.
- The 561 nm excitation laser takes approximately ten minutes to reach full working temperature.

The [Filter Setting] screen appears.

7 Insert all the optical filters in the slots specified in *"Fluorochrome Detection Matrix" (page 204)* and close the flip-up door, then click [Next].



The image displayed shows the optical filter pattern to be installed.

The emission spectra of the fluorochrome labels you wish to detect determine the optical filters needed for an experiment.

For a list of fluorochromes versus optical filters and the corresponding optical filter channels for each model, see "Fluorochrome Detection Matrix" (page 204).

Notes

- Make sure that the correct filter is inserted in each slot for the recommended filter pattern. Correct data cannot be obtained if a filter is inserted in the wrong slot.
- Exercise extreme care when handling the optical filters. The surfaces of each optical filter must be free of dust, smudges, fingerprints, and scratches for accurate data acquisition and cell sorting operation.

  For details about handling optical filters, see "Cleaning and Handling of Optical Filters" (page 186).

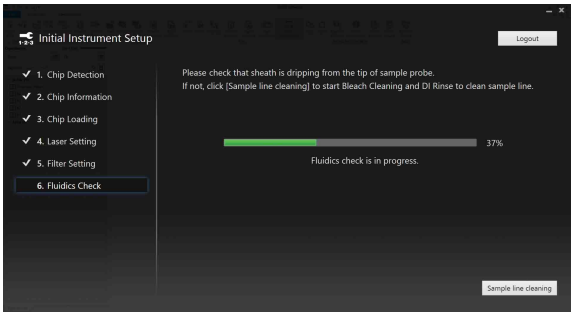
RESERVE slots

- On the following models, dummy filters are installed in the RESERVE slots.
  SH800AC, SH800BC, SH800DC, SH800ZA, SH800ZB, SH800ZD, SH800ZH, SH800ZAP, SH800ZBP, SH800ZDP, SH800ZHP
- On the following models, filters not used in the current optical filter pattern should be stored in the RESERVE slots. Filter pattern 1 can also be used when the 405 nm laser is turned off to make efficient use of the six fluorescence detection channels.
  SH800CC, SH800EC, SH800FC, SH800ZC, SH800ZE, SH800ZF, SH800ZG, SH800ZCP, SH800ZEP, SH800ZFP, SH800ZGP

  For details about filter pattern 1 and 2, see "Optical Filter Patterns" (page 202).

8 Click [Next].

The [Fluidics Check] screen appears.

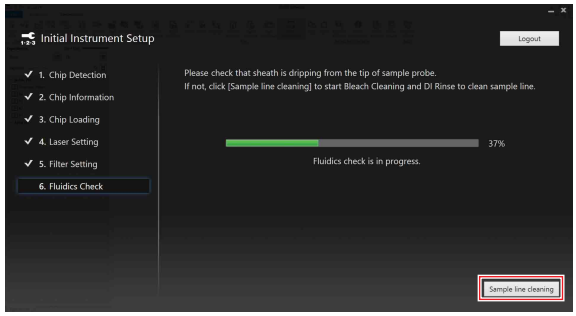

Initial Instrument Setup
Logout
1. Chip Detection
2. Chip Information
3. Chip Loading
4. Laser Setting
5. Filter Setting
6. Fluidics Check
Please check that sheath is dripping from the tip of sample probe.
If not, click [Sample line cleaning] to start Bleach Cleaning and DI Rinse to clean sample line.
37%
Fluidics check is in progress.
Sample line cleaning

When fluidics check starts, sheath fluid droplets appear from the tip of the sample probe. If droplets do not appear, the sample line or the sample probe may be clogged. Click [Sample line cleaning] on the bottom right of the screen to clean the sample line. When the fluidics check is completed, the [Auto Calibration] screen appears.

Cleaning the Sample Line

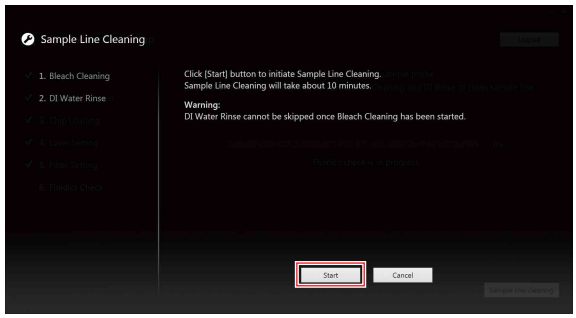
You can clean the sample line before running automatic calibration.

1 Click [Sample line cleaning] on the [Fluidics Check] screen of the [Initial Instrument Setup] wizard.


Initial Instrument Setup
Logout
1. Chip Detection
2. Chip Information
3. Chip Loading
4. Laser Setting
5. Filter Setting
6. Fluidics Check
Please check that sheath is dripping from the tip of sample probe.
If not, click [Sample line cleaning] to start Bleach Cleaning and DI Rinse to clean sample line.
37%
Fluidics check is in progress.
Sample line cleaning

The [Sample Line Cleaning] wizard appears.

2 Click [Start] to begin cleaning the sample line.


Sample Line Cleaning
1. Bleach Cleaning
2. DI Water Rinse
Click [Start] button to initiate Sample Line Cleaning.
Sample Line Cleaning will take about 10 minutes.
Warning:
DI Water Rinse cannot be skipped once Bleach Cleaning has been started.
Start
Cancel

Follow the on-screen instructions.

In sample line cleaning, the fluidics system is cleaned using a sodium hypochlorite solution (bleach cleaning) and DI water, in that order.

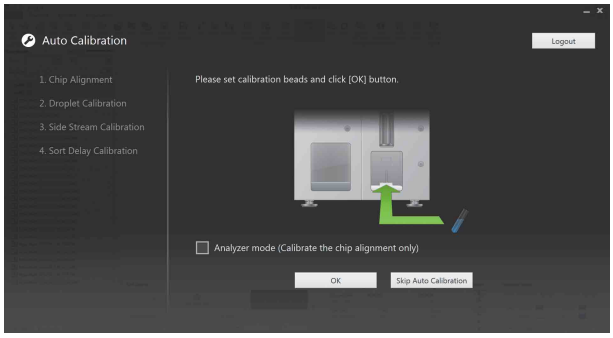
Tips

- You can skip each cleaning step by clicking [Skip], but cleaning with DI water after cleaning with bleach cannot be skipped.
- You can also clean the system again after running the cleaning steps by clicking [Cleaning again].
- For details about cleaning using Sodium Hypochlorite solution, see *"Cleaning the Sample Fluidics System using Bleach" (page 152).*

When sample line cleaning finishes, the [Fluidics Check] screen appears. When the fluidics check is completed, the [Auto Calibration] screen appears.

Automatic Calibration

After configuring the excitation lasers and emission detection optical filters, the startup procedure automatically performs sorting chip alignment, droplet calibration, sort delay calibration, and side stream calibration using automatic setup beads on the [Auto Calibration] screen.



Tips

- You can click [Skip Auto Calibration] to bypass automatic calibration and proceed directly to the SH800 Software main window. Bypassing automatic calibration is useful for quickly accessing the main window to perform analysis of existing data. Do not bypass automatic calibration if you plan to run any new samples.
- You can align the sorting chip and calibrate the sorting system later using [Chip Alignment] and [Sort Calibration], respectively, on the [Cytometer] tab of the ribbon from the main window.

1 Insert 1 ml or more of undiluted, automatic setup beads in a sample tube, and place the tube in the corresponding sample tube holder.

For details about sample tubes, sample tube holders, and automatic setup bead preparation, see "Preparing a Sample" (page 31).

2 Press the sample loader door button to open the sample loader door, and place the sample tube holder in the sample loader.

Align the D-shaped notch on the bottom of the sample tube holder with the slot in the top of the agitation unit when loading the sample tube holder.

Notes

- Always wear laboratory-use gloves, mask, protective goggles, and other protective clothing when handling sample fluid to provide protection against biohazards.
- Also, take care when loading the sample tube in the tube holder to avoid sample fluid spills and breakage of the sample tube.
- Before using a tube, check that there is no dust or other foreign matter in the tube.
- Do not subject the instrument to vibration during operation. Shock or vibration may adversely affect alignment and calibration.

3 Press the sample loader door button to close the sample loader door.

Tip

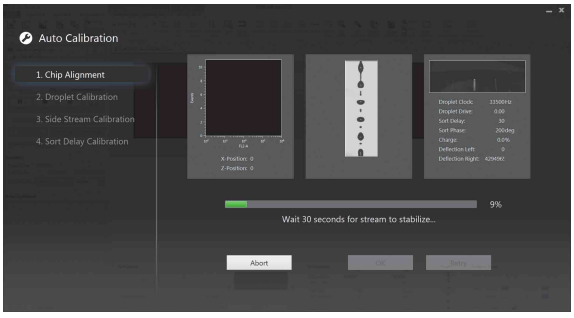
The sample loader door will close automatically, if you leave the door open, when you click [OK] in SH800 Software in the next step to begin calibration.

4 Click [OK] on the [Auto Calibration] screen.

Tip

If you are planning to analyze samples without sorting them, you can select the [Analyzer mode (Calibrate the chip alignment only)] checkbox and click [OK] to bypass sorting calibration. This displays the main window in Analyzer mode.

The automatic calibration sequence starts.
A progress bar displays the status at each stage of the calibration process.



As automatic calibration proceeds, a histogram for the automatic setup beads, the droplet camera image and the side stream monitor image are displayed.

Tip

During automatic calibration, you can click [Abort] to return to the previous screen.

When automatic calibration finishes, [Abort] is disabled, and [Retry] and [OK] are enabled. Clicking [Retry] restarts the automatic calibration procedure.

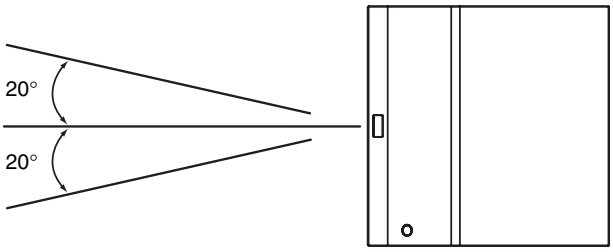
5 When calibration finishes, click [OK].

The [Create Experiment] window appears for building a new experiment.

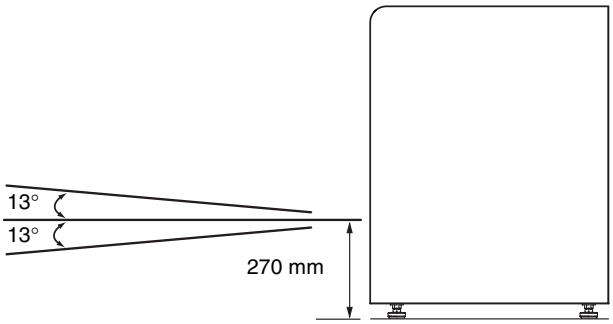
Note

During automatic calibration, precision measurement is performed using the side stream monitor. Accordingly, do not place any bright light sources (halogen lamps, for example) within the following angular ranges. Doing so may cause automatic calibration to malfunction.

Top view



Side view



Creating an Experiment

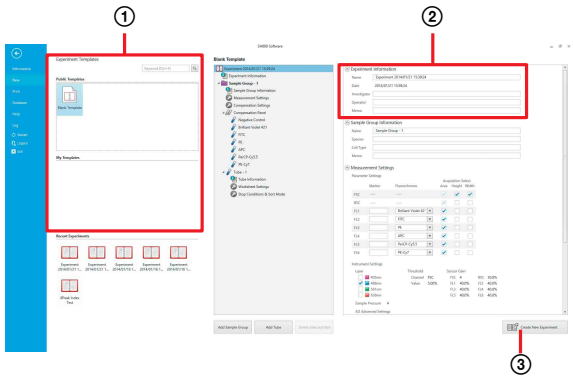
When calibration finishes, you create an experiment that is used to acquire data from samples in the Create Experiment window.

Creating a New Experiment

This section describes how to create a new experiment using existing templates and/or recent experiments.

1 If the [Create Experiment] window is not displayed, click [New] on the [File] tab of the ribbon.

2 Create an experiment.



① Select a template.
Clicking a template or an experiment on the left displays the structure of the selected experiment on the right side of the window.

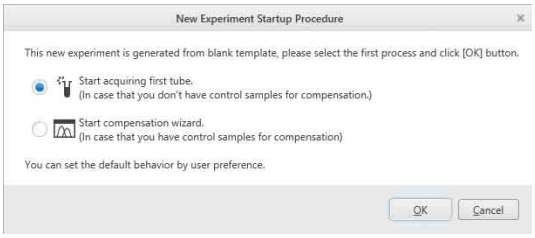
For details about changing the structure of an experiment, see "Creating a Customized Experiment" (page 70).

② Enter a name for the experiment in [Name].
You can configure sample groups, sample tubes, pulse parameters, and other settings for data acquisition.

For details about each item, see "[Create Experiment] Window" (page 111).

③ Click [Create New Experiment].
If "Blank Template" was selected, the [New Experiment Startup Procedure] dialog appears. Proceed to step **3**.
If an existing template or experiment was selected, the main window appears. See *"Acquiring Data" (page 49).*

3 Select the action to perform, then click [OK].

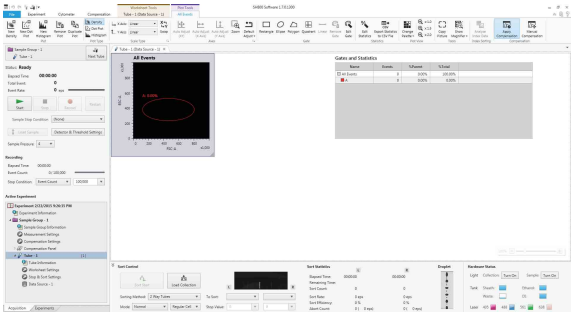

New Experiment Startup Procedure
This new experiment is generated from blank template, please select the first process and click [OK] button.
Start acquiring first tube.
(In case that you don't have control samples for compensation.)
Start compensation wizard.
(In case that you have control samples for compensation)
You can set the default behavior by user preference.
OK
Cancel

[Start acquiring first tube]: Displays the main window and assigns the first tube in preparation for data acquisition.

[Start compensation wizard]: Displays the [Compensation Wizard] to record control tubes for fluorescence compensation.

For details about the [Compensation Wizard], see "Performing Fluorescence Compensation" (page 61).

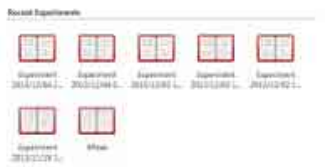
The experiment is created, and the main window appears.
The created experiment is automatically made the active experiment, and the first tube is automatically assigned as the active tube.



To create an experiment using an existing experiment

1 In step **2**-① of the procedure above, select an experiment you want to use from [Recent Experiments] instead of a template.

[Recent Experiments] displays the most recently used experiments in reverse chronological order.



2 Check the experiment details, and enter a name for the experiment in [Name] under [Experiment Information].

3 Click [Create New Experiment].

The experiment is created, and the main window appears.

To save an experiment as a template

You can save an experiment as a shared template (Public Templates) or a private template (My Templates).

For details, see "Saving an Experiment as a Template" (page 78).

Acquiring Data

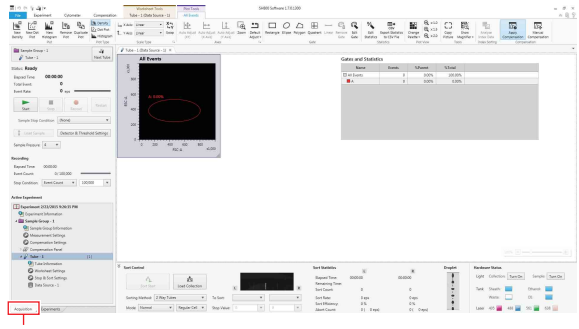
After an experiment is created, you acquire and record data for samples in the experiment from the main window. Recorded data is displayed in real-time, and can also be exported for analysis at a later time.

Before starting acquisition

Check the following items:

- Check the fluid levels in each of the tanks.
- Check that the main unit and fluidics cart are connected correctly.

You control data acquisition and recording in the [Acquisition] tab of the Experiment/Acquisition control pane.



[Acquisition] tab

1 Insert sample fluid in a sample tube, and place the tube in the corresponding sample tube holder.

For details about sample tubes and sample tube holders, see "Preparing a Sample" (page 31).

2 Place the sample tube holder in the sample loader.

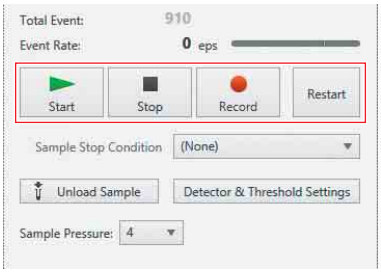
If the sample loader door is closed, press the sample loader door button to open the sample loader door.

3 Press the sample loader door button to close the sample loader door.

Tip

The sample loader door will close automatically, if you leave the door open, when you click Start (Start) in SH800 Software to start acquisition.

4 Specify the measurement stop condition in [Sample Stop Condition].



The following options are available.

- None (data acquisition will stop automatically when the number of detected events exceeds 100,000,000 or the elapsed time exceeds 12:00:00, whichever occurs first)
- Recording (stops data acquisition automatically when the recording stop condition is satisfied)
- Sorting (stops data acquisition automatically when the sorting stop condition is satisfied)
- Recording & Sorting (stops data acquisition automatically when both the recording and sorting stop conditions are satisfied)

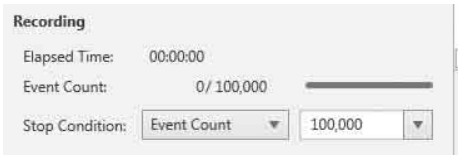
5 Configure the detector settings.

Adjust the gain, threshold, and other settings.

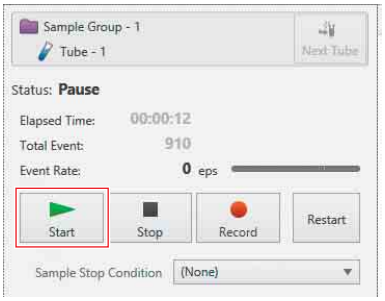
For details, see "Configuring Detector Settings" (page 79).

6 Specify the recording stop condition in [Stop Condition] under [Recording].

You can select [None], [Event Count], or [Elapsed Time] for the recording stop condition, and select a value from the drop-down list.
You can also enter a value directly from the keyboard.



7 Click Start (Start).



Sample fluid starts flowing from the sample tube into the sorting chip, and data acquisition starts.

8 Click (Record).

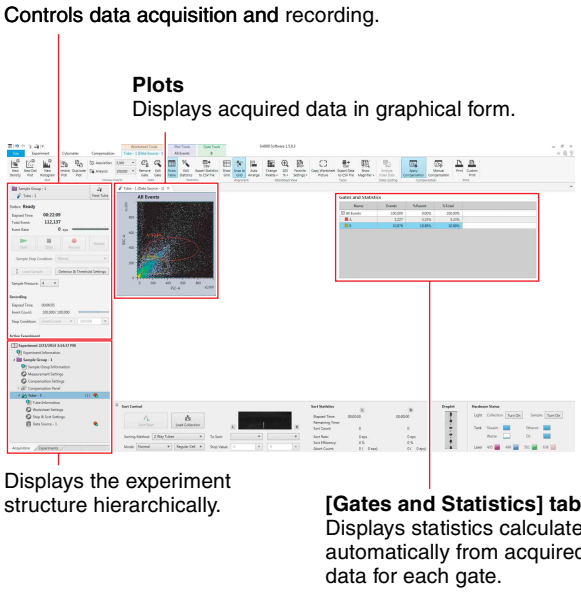
Data recording starts.
If an automatic stop condition was specified, data acquisition and recording stops automatically when the stop condition has been satisfied.

9 If an automatic stop condition was not specified (None), click (Stop) to stop data acquisition and recording.

The sample tube unloads automatically.

[Acquisition] Tab Main Functions

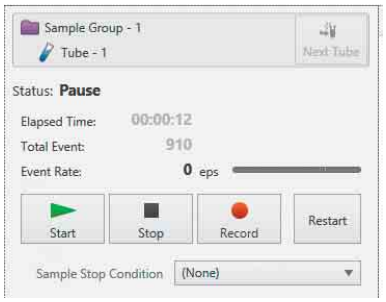
During data acquisition, the data is displayed on plots and in the [Gates and Statistics] table on the worksheet. You use the buttons on the [Acquisition] tab, as required, to control data acquisition while monitoring the data acquisition status on the worksheet. You can change the type of plots, edit gates on plots, and customize the content displayed in the [Gates and Statistics].


Controls data acquisition and recording.
Plots
Displays acquired data in graphical form.
Displays the experiment structure hierarchically.
[Gates and Statistics] table
Displays statistics calculated automatically from acquired data for each gate.

Controlling Data Acquisition and Recording

After data acquisition starts, the elapsed time since the start of data acquisition, the total number of events acquired, and the event rate measured in events/second (eps) are displayed on the [Acquisition] tab.
You control acquisition, as required, while monitoring the acquisition status.

For details about the displayed items, see "[Acquisition] Tab (control pane)" (page 132).



(Start): Starts/resumes data acquisition. It does not start data recording.
(Pause): Pauses data acquisition.
The elapsed time continues counting while acquisition is paused.
(Stop): Stops data acquisition manually.
(Record): Records the acquired data.
(REC stop): Stops data recording manually.
(Restart): Resets the total events and elapsed time counters to 0 and restarts data acquisition.

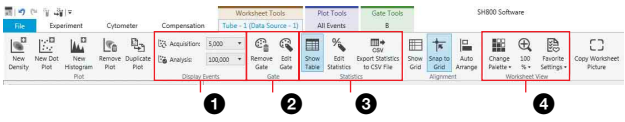
Tip

If an automatic stop condition has been specified, data acquisition and/or recording stops automatically when the corresponding stop condition has been satisfied.

Customizing the Worksheet

You can change the type of plots, modify gates on plots, and customize the content in the [Gates and Statistics] table displayed on the worksheet during data acquisition. You customize the items on the worksheet using the tabs on the ribbon.

Example: [Worksheet Tools] tab



❶ [Display Events]
Sets the number of events displayed on plots.

❷ [Gate]
Tools for editing gates.

❸ [Statistics]
Shows/hides the [Gates and Statistics] table, customizes the content displayed in the table, and exports the table as a CSV file.

❹ [Worksheet View]
Changes the view and scale of plots on the worksheet.

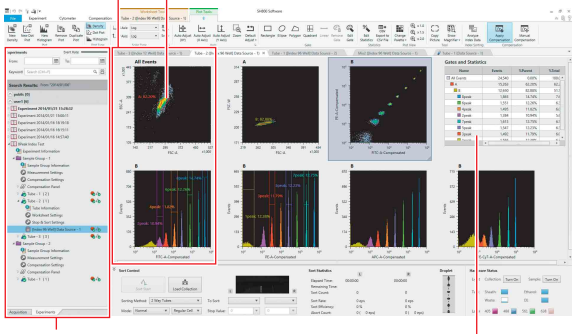
For details, see "[Worksheet Tools] Tab (ribbon)" (page 125).

Analyzing Data

After data acquisition finishes, you analyze the acquired data events. The most recent data is displayed on the worksheet immediately after acquisition finishes. To analyze data for a past experiment, search for and select the target experiment on the [Experiments] tab of the Experiment/Acquisition control pane.

Plots
Displays acquired data in graphical form.
Plots can be added/edited.

Gates
Groups events with common characteristics, isolating them from all other events.



[Experiments] tab
Displays experiments that can be selected for analysis.

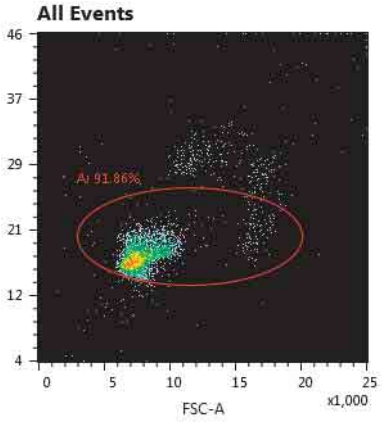
[Gates and Statistics] table
Displays statistics calculated automatically from acquired data for each gate. You can customized the statistics displayed in the table.

This section describes how to add plots to the worksheet, how to add gates to a plot, and how to display statistics for the gates.

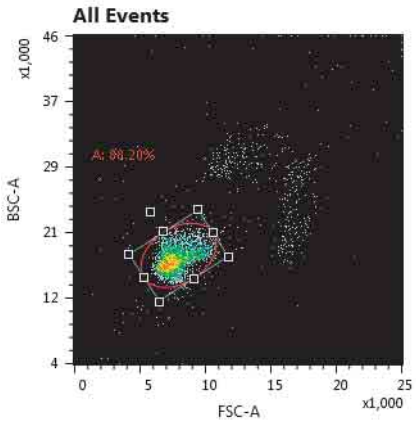
For details, see "Analysis" (page 83).

1 Select the [All Events] plot on the worksheet.

The [All Events] plot is the top level in the hierarchy. All plots for gates are derived from [All Events]. If necessary, adjust the axes scales using the tool buttons in the [Axes] group on the [Plot Tools] tab of the ribbon.



2 Adjust the gate size and position to surround the target population.



3 Double-click the gate in the plot to add a child plot for that gate.

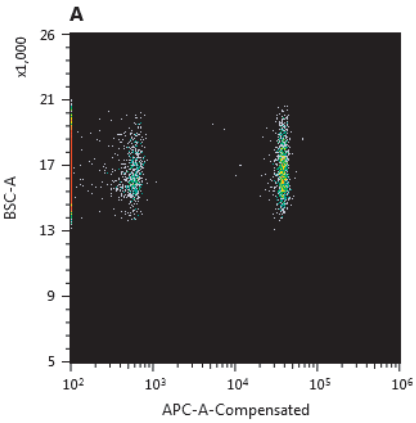
You can also add a child plot from the context menu by right-clicking the gate in the [Gates and Statistics] table.

Tips

- You can add a new plot using the tool buttons in the [Plot] group *(page 125)* on the [Plot Tools] tab or the [Worksheet Tools] tab of the ribbon.
- You can change the type of the plot using the tool buttons in the [Plot Type] group *(page 129)* on the [Plot Tools] tab of the ribbon.

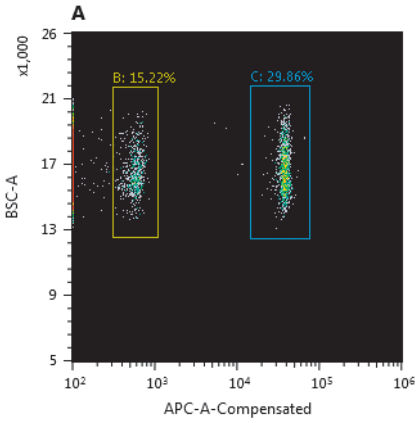
4 Click the axis labels and select the parameters to be displayed from the popup menu.

Adjust the axes scales to display the events on the plot, as required.



5 Select a gate type in the [Gate] group of the [Plot Tools] tab, and draw gates around both of the populations in the child plot.

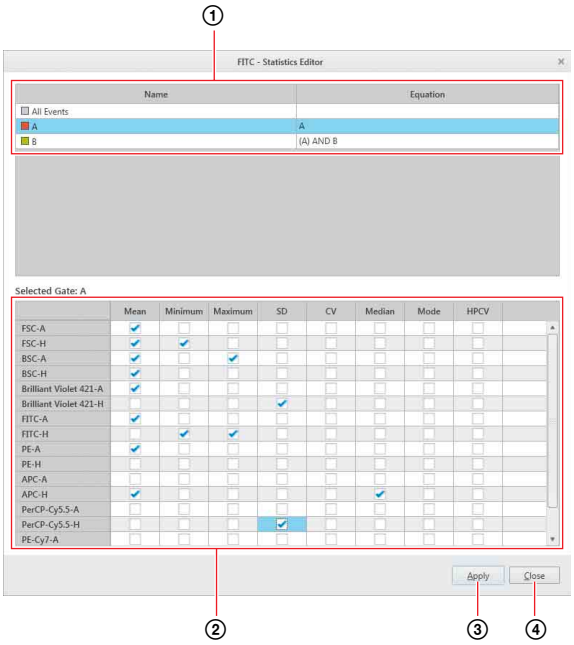
The gates are automatically named in alphabetical order. You can change the name and color of gates in the [Property Window] dialog by right-clicking a gate and selecting [Properties] from the context menu.



6 Click [Edit Statistics] in the [Statistics] group on the [Worksheet Tools] tab or [Plot Tools] tab.

The [Statistics Editor] dialog appears.

7 Select the statistics to display in the [Gates and Statistics] table.

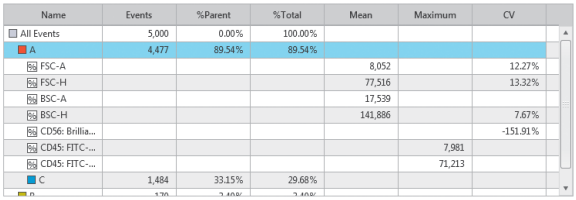


① Select a gate.

② Place check marks in the checkboxes for the statistics to display.

③ Click [Apply] to update the [Gate and Statistics] table.

④ Click [Close] to close the dialog.
The selected statistics are displayed in the [Gates and Statistics] table.



Sorting

Once events have been isolated on plots using gates, the same events can be sorted into different collection tubes for further analysis. Sorting enables the targeted cells to be recovered from a sample to increase the concentration of the cells or to isolate cells for increased purity.

Supported sorting methods for sorting chip nozzle diameter

The sorting methods that can be used vary depending on the nozzle diameter of the sorting chip and the size of cells.

SH800 and SH800Z

Sorting method		2 Way Tubes		8 Well Strips		Slides	
Cell size settings		Regular Cell	Large Cell	Regular Cell	Large Cell	Regular Cell	Large Cell
Sorting chip nozzle	100 µm	Yes	Yes	Yes	Yes	Yes	Yes
	130 µm	Yes	–	–	–	–	–

SH800ZP

Sorting method		2 Way Tubes		96 Well Plate		384 Well Plate	
Cell size settings		Regular Cell	Large Cell	Regular Cell	Large Cell	Regular Cell	Large Cell
Sorting chip nozzle	100 µm	Yes	Yes	Yes	Yes	Yes	Yes
	130 µm	Yes	–	–	–	–	–

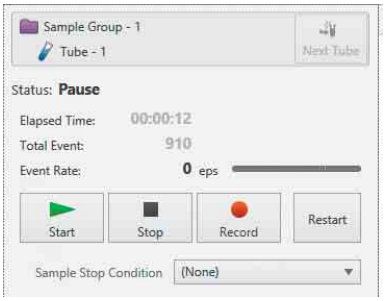
Notes

- Sorting into 8-well strips, multi-well plates, or onto glass slides is not supported when using the 130 µm sorting chip.
- [Large Cell] is an assistive mode for the sorting of large-sized cells when using the 100 µm sorting chip. Use it if you are having trouble sorting large cells. However, this function does not guarantee complete accuracy in sorting large cells. A rough guide for the size of large cells for which to use the [Large Cell] setting is 15 µm or larger.

Two-way Tube Sorting

1 Check that data acquisition and recording are stopped or paused.

If data acquisition is in progress, click the Stop (Stop) to stop data acquisition/recording or Pause (Pause) to pause data acquisition/recording.

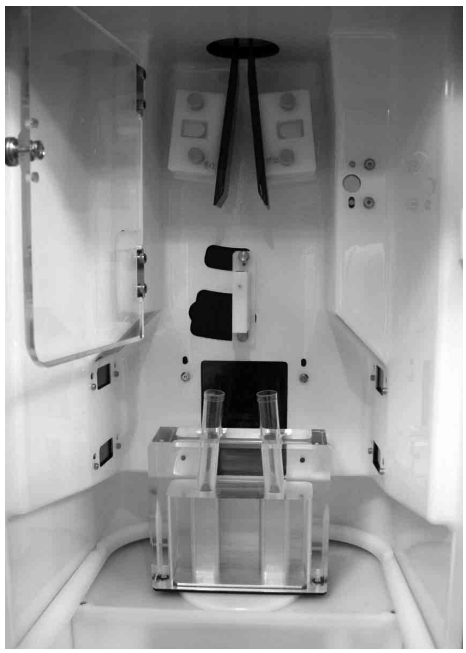


2 Place the collection tubes into the corresponding holder.

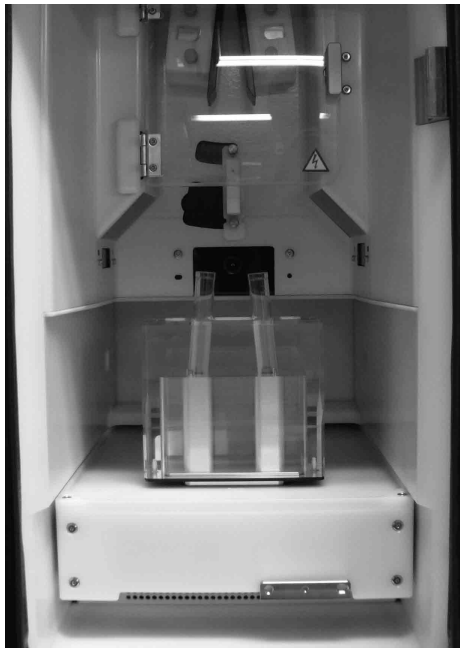
For details, see "Preparing Collection Tubes" (page 33).

3 Open the sorting area door, place the holder on the collection stage, and close the door.

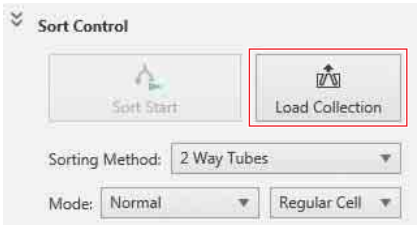
SH800



SH800ZP



4 Click [Load Collection] in the [Sort Control] pane.



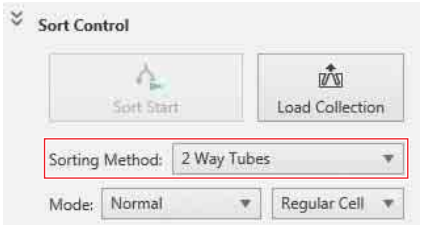
The collection tube device is loaded.

Note

Do not open the sorting area door during sorting.

5 Select [2 Way Tubes] in [Sorting Method].

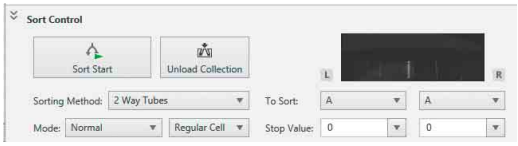
Always select the sorting method matching the collection tube device loaded on the collection stage.



The sorting parameters vary depending on the selected sorting method.

6 Set each parameter.

For details about each item, see "Sorting Control Pane" (page 136).

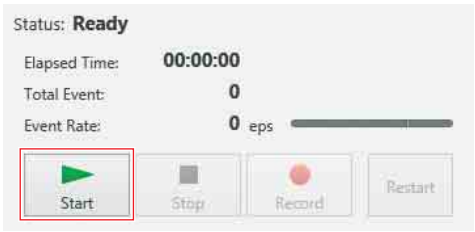


[Mode]: Selects the sorting mode.

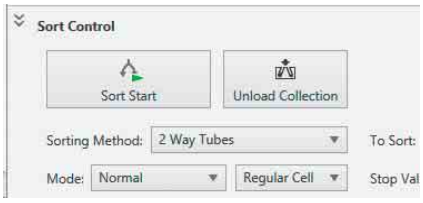
[To Sort]: Selects the gates for the target events to sort into the left and right collection tubes.

[Stop Value]: Specifies the event count conditions to stop recording automatically for each of the left and right collection tubes.
You can select a value or enter a value from the keyboard.
Entering a value of "0" disables the stop condition.

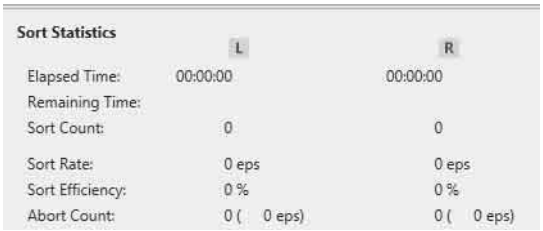
7 Click [Start] or [Resume] in the data acquisition control pane to begin data acquisition.



8 Click [Sort Start] in the [Sort Control] pane to begin sorting.

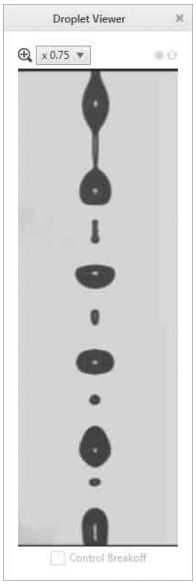


Basic sorting status is displayed, together with progress bars if an automatic stop condition is specified.



You can monitor the side streams and droplet flow in the main window.

Double-clicking the image displays the side streams in a separate window for monitoring sorting.



If an automatic stop condition is configured, a stop message appears when the stop condition is satisfied and sorting stops.

9 Click [OK] to close the confirmation message dialog.

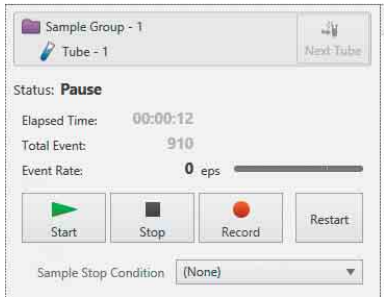
Tip

If an event is detected that falls within both gates selected for the left and right side streams, the event is sorted in the left side stream (left side stream always has precedence).

Sorting onto 8-well Strips (SH800 and SH800Z)

1 Check that data acquisition and recording are stopped or paused.

If data acquisition is in progress, click the Stop (Stop) to stop data acquisition/recording or Pause (Pause) to pause data acquisition/recording.

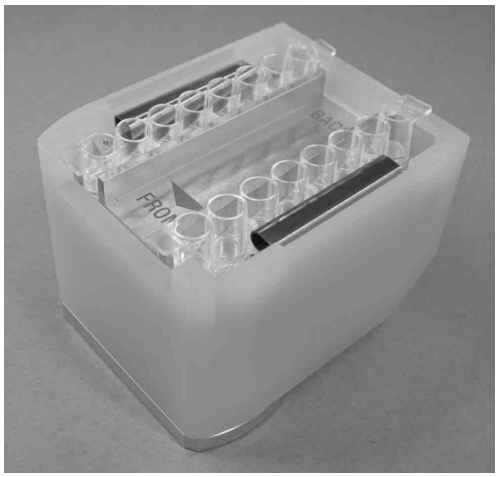


2 Place the 8-well strips or glass slides onto the corresponding holder.

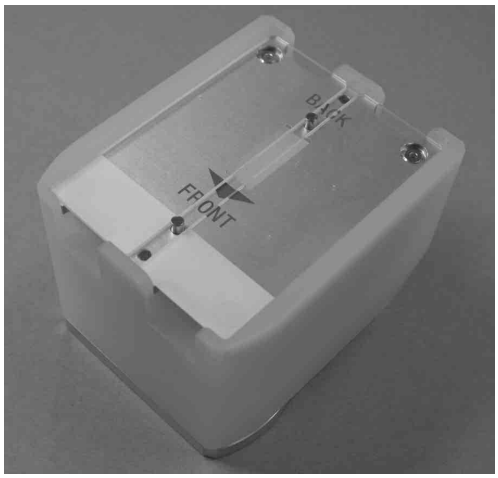
For details, see "Preparing Collection Tubes" (page 33).

3 Open the sorting area door, place the holder on the collection stage, and close the door.

8-well strips holder



Slide glass holder



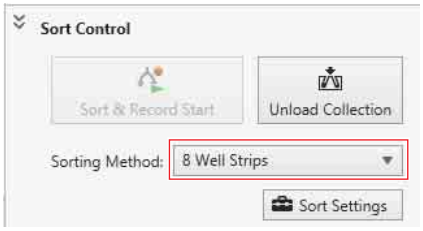
Notes

- Do not sort sample fluid above the permitted volume into the wells and do not overfill the wells with buffer solution to prevent sample fluid overflow from the wells.

- Take care not to spill samples when removing the 8-well strips or glass slides from the multi-well plate holder.
- Ensure that the 8-well strips or slide glass selected in [Sorting Method] is loaded correctly.
- Do not open the sorting area door during sorting.
- Do not attempt to remove the 8-well strips or slide glass while the collection stage is moving.

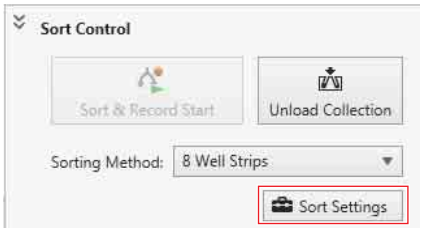
4 Select the appropriate plate sorting method in [Sorting Method].

Always select the sorting method matching the collection tube device loaded on the collection stage.



The sorting parameters vary depending on the selected sorting method.

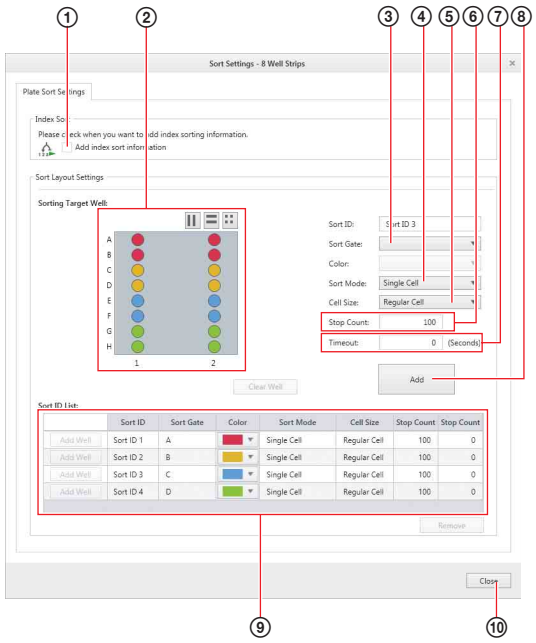
5 Click [Sort Settings].



The [Sort Settings] dialog appears.

6 Configure the settings for sorting into wells on the [Plate Sort Settings] tab.

The description in this section shows an 8-well strip as an example. The operation is the same for glass slides.



① If using index sorting, enable the [Add index sort information] checkbox.
In index sorting, you can associate the cells sorted in each well with the data points for those cells on plots.

For details, see "Index Sorting" (page 101).

② Select the target well to set.
You can select the well using the following buttons or by clicking the well on the palette.
: Select wells in alternate columns.
: Select wells in alternate rows.
: Select wells in groups of four.
You can select multiple wells using the Shift and Ctrl keys.
The ID is displayed in [Sort ID].
You can change the value in [Sort ID] manually.

③ Select the gate whose population you want to sort into the well in [Sort Gate].
You can change the color of the gate in [Color].

④ Select a sorting mode in [Sort Mode].
The default is "Single Cell."
"Single Cell" mode is recommended for reliable sorting.

⑤ Select the size of the cells to be sorted in [Cell Size].
As a rough guide, select [Regular Cell] for cells up to 15 µm in size, and [Large Cell] for larger cells.

Note

When you select [Large Cell], a charge setting suited for sorting larger cells is used. However, this function does not guarantee complete accuracy in sorting large cells.

⑥ Enter the number of events to sort in [Stop Count].

Note

If a value exceeding the values below is entered in [Stop Count], a message appears warning you of the risk that sample fluid may overflow the well (operation does not stop).

Sorting chip	8-well strips	Slides
100 µm nozzle	50,000 events	300 events

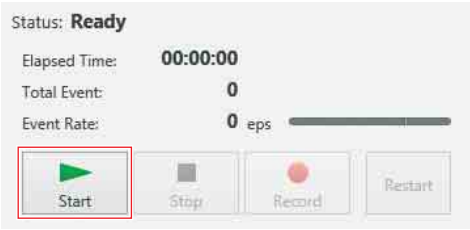
⑦ Enter the timeout time in [Timeout] in units of seconds.

⑧ Click [Add].
The settings are added to [Sort ID List].

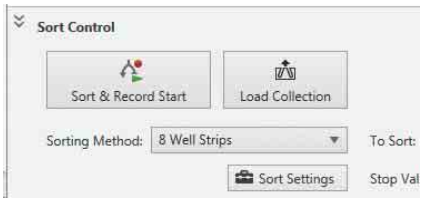
⑨ Repeat steps ② to ⑧ to set parameters for any other wells if necessary.
You can modify the parameters for a well by clicking the corresponding cells in [Sort ID List].

⑩ When finished, click [Close] to close the dialog.

7 Click [Start] or [Resume] in the data acquisition control pane to begin data acquisition.



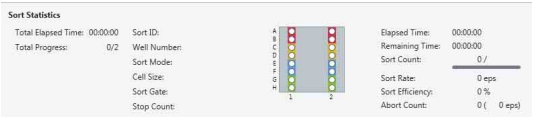
8 Click [Sort Start] in the [Sort Control] pane to begin sorting.



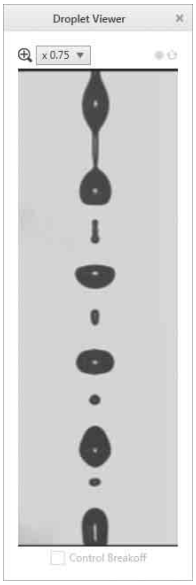
Tip

Starting sorting automatically starts sorting and recording.

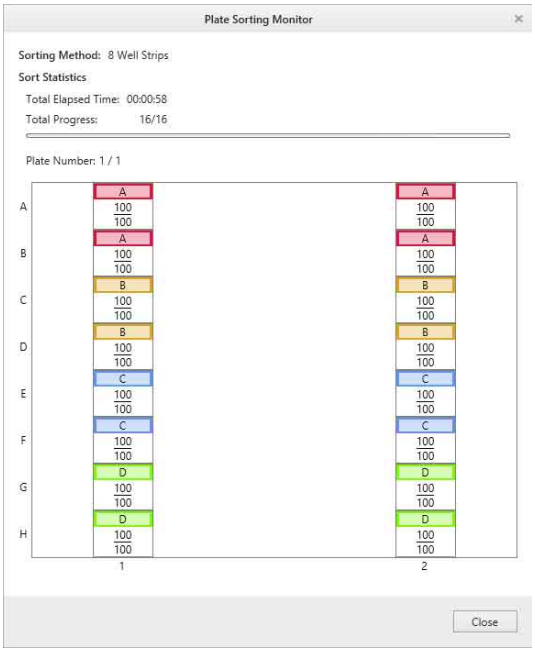
Basic sorting statistics are displayed, together with progress bars if an automatic stop condition is specified.



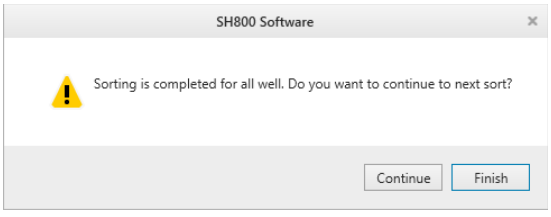
Double-clicking the image displays the side streams in a separate window for monitoring sorting.



Double-clicking the plate image or right-clicking and selecting [Sorting Monitor] from the context menu displays the [Plate Sorting Monitor] window for monitoring progress. You can also display the window by right-clicking a tube in the tube list and selecting [Show Sorting Report] from the context menu.



After one set has been sorted, the following message appears.



9 Click [Finish] to finish sorting.

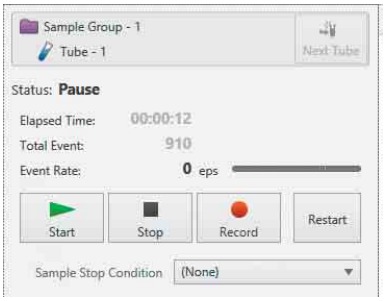
To continue sorting, click [Continue].
When you click [Continue], the collection stage is unloaded, allowing you to place the next set.

Sorting into Multi-well Plates (SH800ZP only)

This section describes the procedure for sorting into a 96-well plate. The procedure is identical for sorting into 6, 12, 24, 48, and 384-well plates.

1 Check that data acquisition and recording are stopped or paused.

If data acquisition is in progress, click the (Stop) to stop data acquisition/recording or (Pause) to pause data acquisition/recording.



2 Attach the splash guard.

Always attach the splash guard when sorting into multi-well plates.

For details, see "Attaching the Splash Guard (for use with multi-well plates) (SH800ZP only)" (page 35).

3 Place the 96-well plate into the corresponding holder.

For details, see "Preparing Collection Tubes" (page 33).

4 Open the sorting area door, place the holder on the collection stage, and close the door.

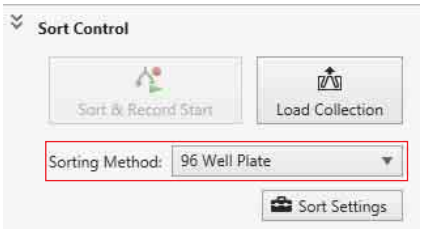
96-well plate holder

Notes

- If the multi-well plate is loaded in the incorrect orientation, correct sorting is not possible. Check the orientation when loading a multi-well plate.
- Do not sort sample fluid above the permitted volume into the wells and do not overfill the wells with buffer solution to prevent sample fluid overflow from the wells.
- Take care not to spill samples when removing the multi-well plate from the multi-well plate holder.
- Securely mount the holder adapter to ensure successful sorting.
- Always load a multi-well plate of the same size as that selected in [Sorting Method].
- Do not open the sorting area door during sorting.
- Do not attempt to remove a multi-well plate while the collection stage is moving.

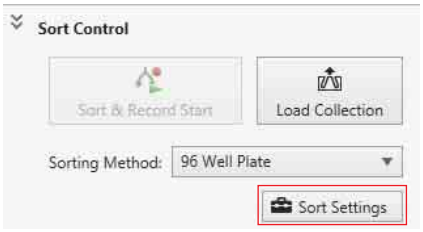
5 Select the appropriate plate sorting method in [Sorting Method].

Always select the sorting method matching the collection tube device loaded on the collection stage.



The sorting parameters vary depending on the selected sorting method.

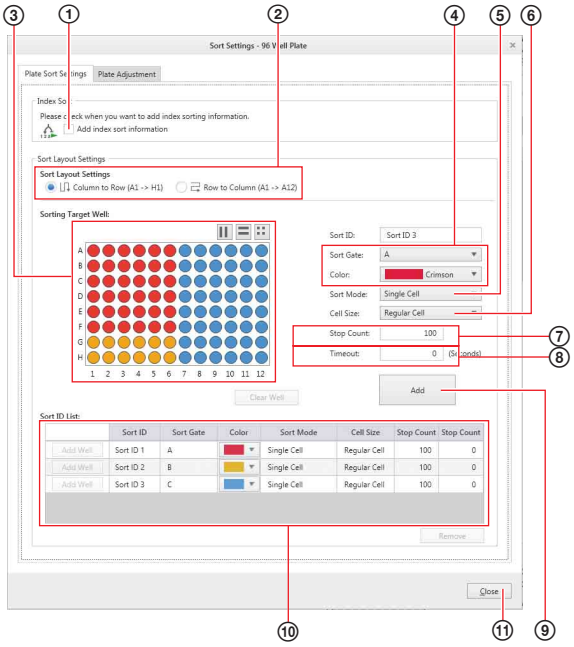
6 Click [Sort Settings].



The [Sort Settings] dialog appears.

7 Configure the settings for sorting into wells on the [Plate Sort Settings] tab.

The description in this section shows a 96-well plate as an example. The operation is the same for all other multi-well plates.



① If using index sorting, enable the [Add index sort information] checkbox.
In index sorting, you can associate the cells sorted in each well with the data points for those cells on plots.

For details, see "Index Sorting" (page 101).

② Select the sorting sequence in [Sort Layout Settings].
- [Column To Row]: Sorting occurs in the sequence A1 → B1 → C1…H1 → A2 → B2…G12 → H12.
- [Row To Column]: Sorting occurs in the sequence A1 → A2 → A3…A12 → B1 → B2…H11 → H12.

③ Select the target well to set.
You can select the well using the following buttons or by clicking the well on the palette.
: Select wells in alternate columns.
: Select wells in alternate rows.
: Select wells in groups of four.
You can select multiple wells using the Shift and Ctrl keys.
The ID is displayed in [Sort ID].
You can change the value in [Sort ID] manually.

④ Select the gate whose population you want to sort into the well in [Sort Gate].
You can change the color of the gate in [Color].

⑤ Select a sorting mode in [Sort Mode].
The default is "Single Cell."
"Single Cell" mode is recommended for reliable sorting.

⑥ Select the size of the cells to be sorted in [Cell Size].
As a rough guide, select [Regular Cell] for cells up to 15 µm in size, and [Large Cell] for larger cells.

Note

When you select [Large Cell], a charge setting suited for sorting larger cells is used. However, this function does not guarantee complete accuracy in sorting large cells.

⑦ Enter the number of events to sort in [Stop Count].

Note

If a value exceeding the values below is entered in [Stop Count], a message appears warning you of the risk that sample fluid may overflow the well (operation does not stop).
96 Well Plate: 50,000 events

⑧ Enter the timeout time in [Timeout] in units of seconds.

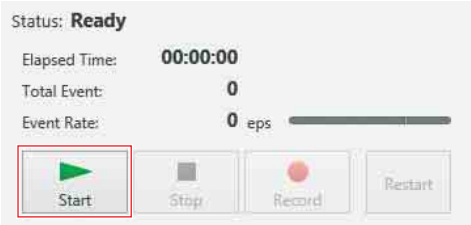
⑨ Click [Add].
The settings are added to [Sort ID List].

⑩ Repeat steps ③ to ⑨ to set parameters for all wells. You can modify the parameters for a well by clicking the corresponding cells in [Sort ID List].

⑪ When finished, click [Close] to close the dialog.

For details about the [Plate Adjustment] tab, see "Adjusting the Sort Position (SH800ZP only)" (page 99).

8 Click [Start] or [Resume] in the data acquisition control pane to begin data acquisition.

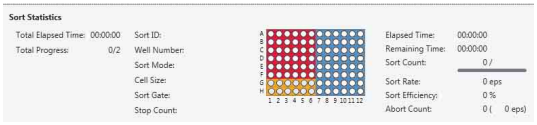


9 Click [Sort Start] in the [Sort Control] pane to begin sorting.

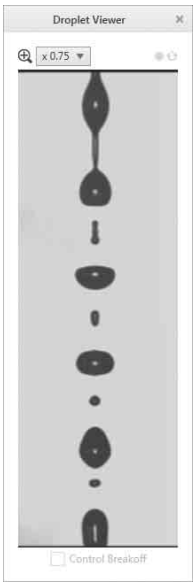
Tip

Starting sorting automatically starts sorting and recording.

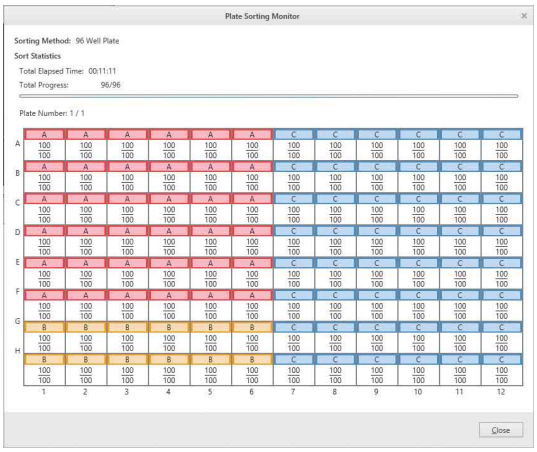
Basic sorting statistics are displayed, together with progress bars if an automatic stop condition is specified.



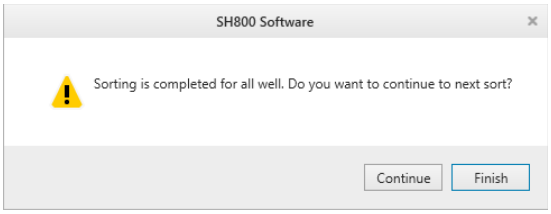
Double-clicking the image displays the side streams in a separate window for monitoring sorting.



Double-clicking the plate image or right-clicking and selecting [Sorting Monitor] from the context menu displays the [Plate Sorting Monitor] window for monitoring progress. You can also display the window by right-clicking a tube in the tube list and selecting [Show Sorting Report] from the context menu.



After one set has been sorted, the following message appears.



10 Click [Finish] to finish sorting.

To continue sorting, click [Continue].
When you click [Continue], the collection stage is unloaded, allowing you to place the next set.

Performing Fluorescence Compensation

Fluorescence compensation is used to remove fluorescence spillover components from each fluorescence detector to obtain the signal for the target fluorochrome only for each detector.
In fluorescence compensation, it is necessary to record a compensation panel comprising an unstained negative control and up to six singly-stained, positive control samples for each fluorochrome.

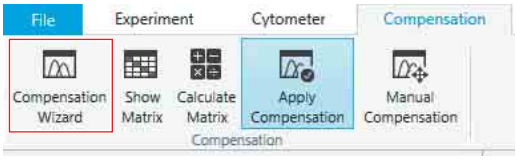
This section describes the fluorescence compensation procedure using the [Compensation Wizard].

For details about using a gate for recording a control tube or adjusting fluorescence compensation manually, see "Adjusting Fluorescence Compensation Manually" (page 81).

Note

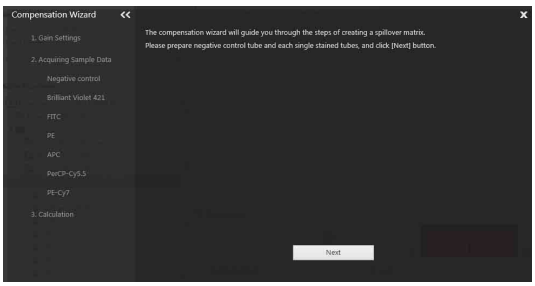
Do not change the gain of the channel detectors while recording the control tubes or samples for a compensation panel. The fluorescence compensation process is valid only for the instrument settings in place when all control tubes in a compensation panel are recorded. If the sensor gain of any detector is changed after recording the control tubes, the fluorescence compensation will no longer be valid and the control tubes will need to be loaded and recorded again.

1 Click [Compensation Wizard] on the [Compensation] tab of the ribbon.



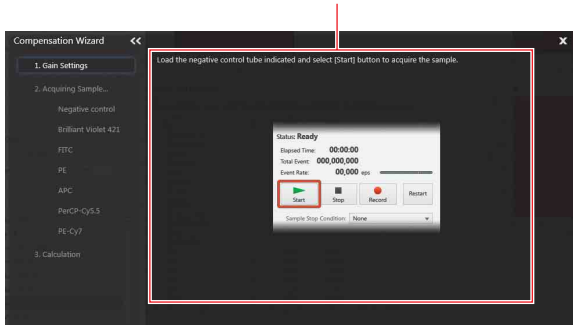
The [Compensation Wizard] launches.

2 Click [Next].



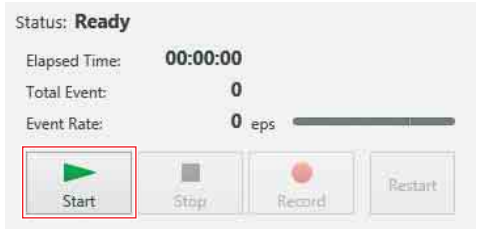
The following screen appears.
Follow the on-screen instructions to record control tubes.

The operating procedure is displayed.



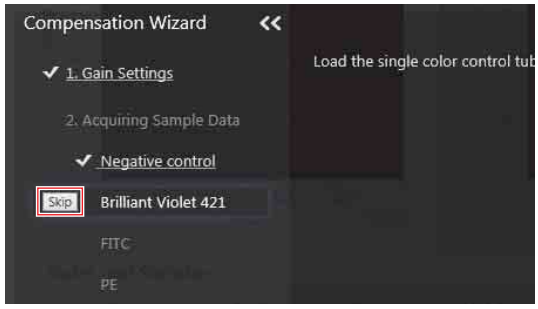
A control tube is automatically assigned as the active tube and the worksheet for the tube opens.

3 Load the control tube displayed in the wizard, then click [Start] on the data acquisition control pane.



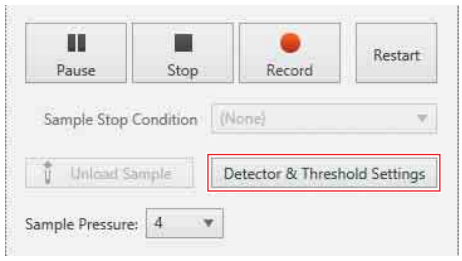
Tip

If, for any reason, you choose not to use a fluorochrome channel, you can click [Skip] to bypass the control tube.



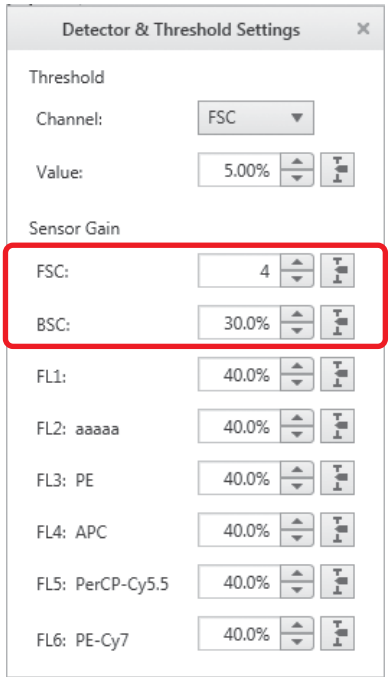
Data acquisition starts for the tube and data points are added to the plots on the worksheet in real-time.

4 Click [Detector & Threshold Settings].



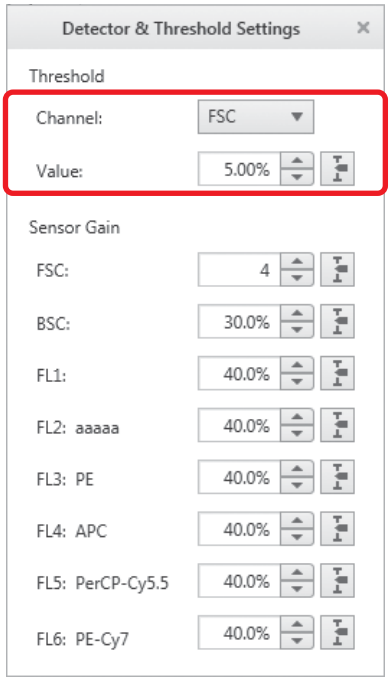
The [Detector & Threshold Settings] dialog appears.

5 Adjust the sensor gain of the FSC and BSC detectors to place the population on scale.

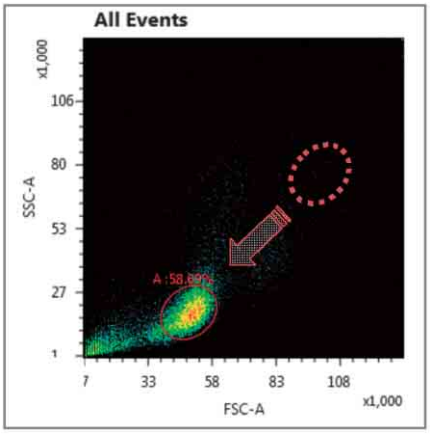


You adjust the gain while viewing the FSC vs. BSC density plot. You can also adjust the axis display scales to display a magnified portion of the plot.

6 Adjust the trigger threshold level to remove background noise without affecting the targeted population.

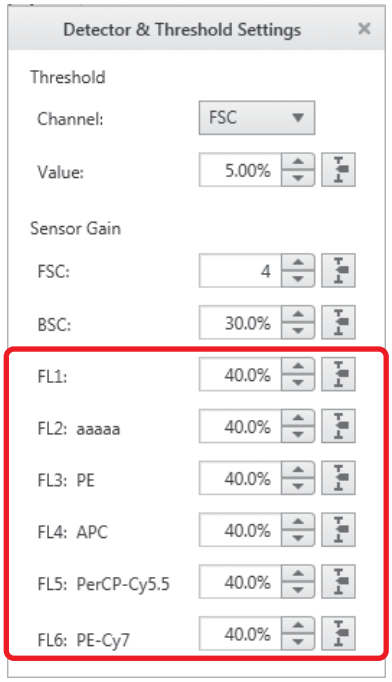


7 Adjust the size and position of the gate automatically added to the plot to encompass the desired population, then click [Next] in the [Compensation Wizard].

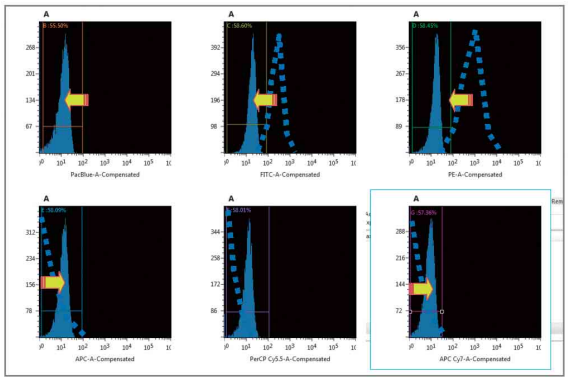


8 Adjust the gain levels of the FL1 to FL6 fluorescence channels to place the negative populations on scale in all channels.

You adjust the gain while viewing the histogram plots for each fluorescence channel.

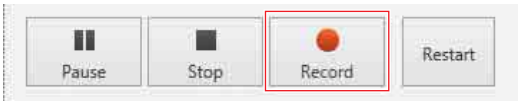


The negative peaks represent the fluorescence signals detected due to cellular autofluorescence. The negative peaks should be on scale, and not wedged up against the axis in all channels.



9 After adjusting the detector gain settings, click [Next].

10 Click [Record] in the data acquisition control pane.

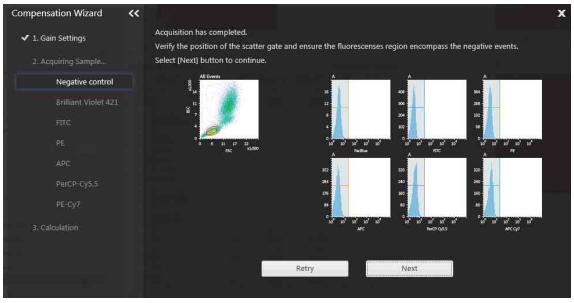


The SH800 starts recording the data acquired for the negative control tube. Recording stops automatically after satisfying an internal record stop condition when sufficient data has been recorded.

Tip

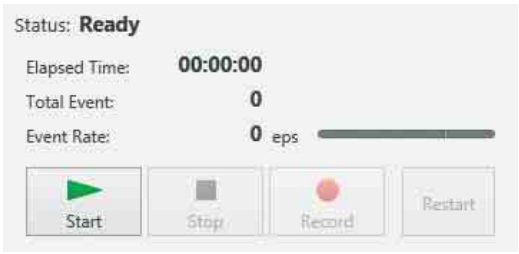
You can stop data acquisition and recording manually using [Stop] if a sufficient number of events have been recorded and the plot view has stabilized.

11 Click [Next].



Data acquisition and recording for the negative control tube is completed.
You are then prompted to load and record the positive control tubes.

12 Load the next control tube displayed in the wizard, then click [Start] on the data acquisition control pane.



The positive controls must be loaded in the sequence displayed in the Experiment Explorer, as prompted by the [Compensation Wizard].

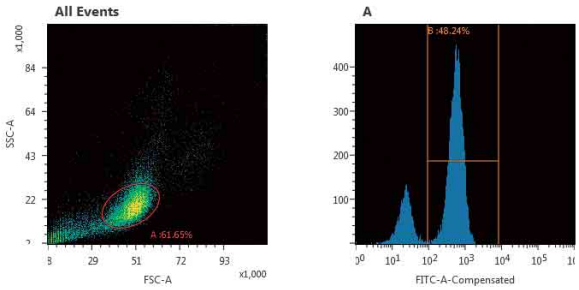
13 Check that data acquisition has started, then click [Record] in the data acquisition control pane.

Recording stops automatically after satisfying an internal record stop condition when sufficient data has been recorded. The positive population is displayed on the histogram plot.

Tip

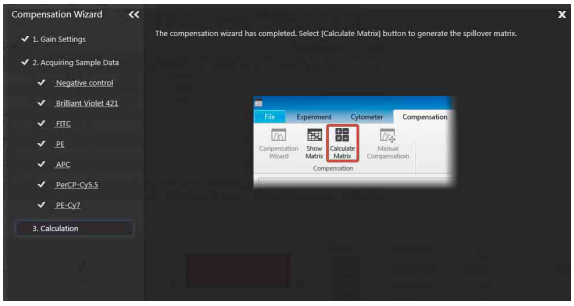
You can stop data acquisition and recording manually using [Stop] if a sufficient number of events have been recorded and the plot view has stabilized.

14 Check the position of the gate on the density plot and the gate for the positive population on the histogram, adjust the gate if necessary, then click [Next].



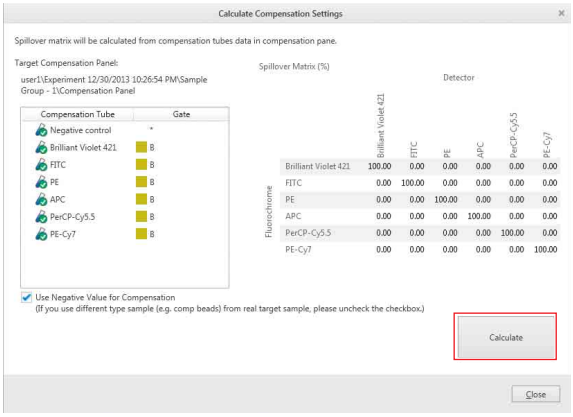
15 Repeat steps **11** to **14** to record each of the control tubes in sequence.

16 After recording all control tubes, click [Calculate Matrix] on the [Compensation] tab of the ribbon.



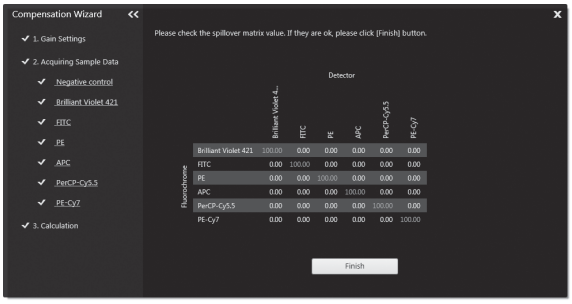
The [Calculate Compensation Settings] dialog appears.

17 Check the target compensation panel, then click [Calculate].



The spillover matrix and the negative fluorescence compensation values are calculated and then displayed.

18 Check the compensation matrix values.



19 Click [Finish] to exit the [Compensation Wizard].

Flushing the Fluidics System

When measuring samples, the sorting chip, sample line, and sample probe should always be flushed with sheath fluid after unloading a sample tube and before loading the next sample tube to eliminate carryover contamination between samples.

1 Check that data acquisition and recording are stopped.

The fluidics system cannot be washed during data acquisition.

2 Click [Probe Wash] in the [Cleaning] group on the [Cytometer] tab of the ribbon.

The sorting chip, sample line, and sample probe are flushed with sheath fluid.

For details about cleaning manually using bleach, see "Cleaning the Sample Fluidics System using Bleach" (page 152).

Shutdown

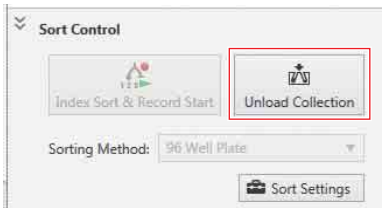
Shut down the SH800 when finished measurement for the day using the following procedure. The fluidics system should be cleaned according to the instructions in the Shutdown Wizard before shutting down the SH800 main unit.

The Shutdown Wizard describes the bleach cleaning procedure. For details, see "Cleaning the Sample Fluidics System using Bleach" (page 152).

Note

Always leave a sorting chip in the loader when shutting down. The chip seals the inlet ports when the SH800 is not in use.

1 Click [Unload Collection] to unload the collection stage and remove the collection tubes.



Tip

Remove the splash guard when unloading multi-well plates after sorting is finished.

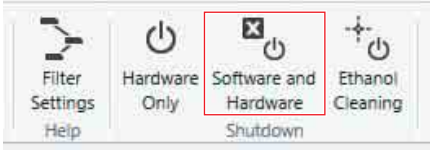
2 Clean the sample loader, sorting area, and the collection stage using ethanol to prevent salt precipitation within the unit and to prevent biological contamination.

For details about cleaning, see "General Cleaning" (page 153).

Note

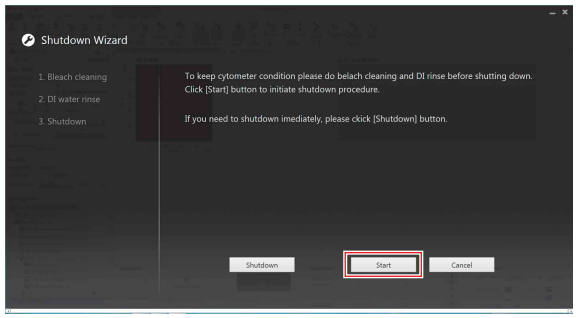
After cleaning, the fluidics lines are filled with DI water to prevent the formation of salt deposits. Running data acquisition in this state may damage cells.

3 Click [Software and Hardware] in the [Shutdown] group on the [Cytometer] tab of the ribbon.



The Shutdown Wizard appears.

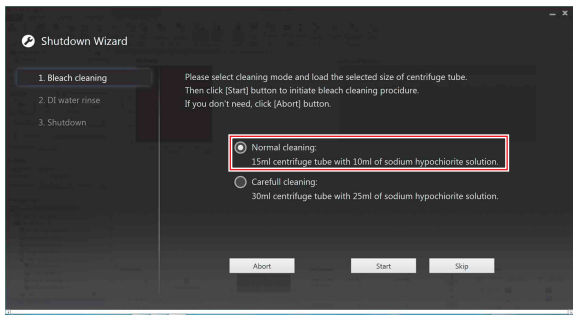
4 Click [Start].


Shutdown Wizard
1. Bleach cleaning
2. DI water rinse
3. Shutdown
To keep cytometer condition please do belach cleaning and DI rinse before shutting down.
Click [Start] button to initiate shutdown procedure.
If you need to shutdown imediately, please ckick [Shutdown] button.
Shutdown
Start
Cancel

Tip

If you click [Shutdown], the SH800 shuts down without doing any cleaning.

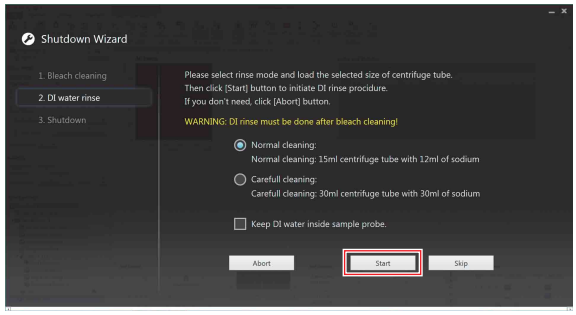
5 Select the sodium hypochlorite cleaning sample tube size and click [Start].


Shutdown Wizard
1. Bleach cleaning
2. DI water rinse
3. Shutdown
Please select cleaning mode and load the selected size of centrifuge tube.
Then click [Start] button to initiate bleach cleaning procidure.
If you don't need, click [Abort] button.
Normal cleaning:
15ml centrifuge tube with 10ml of sodium hypochiorite solution.
Carefull cleaning:
30ml centrifuge tube with 25ml of sodium hypochiorite solution.
Abort
Start
Skip

Bleach cleaning starts.
When bleach cleaning finishes, flush the system using DI water.

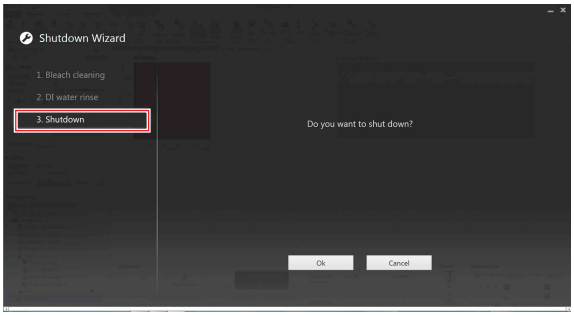
6 Select the DI water cleaning sample tube size and click [Start].

To shutdown the instrument with the sample probe filled with DI water, place a check mark in [Keep DI water inside sample probe].


Shutdown Wizard
1. Bleach cleaning
2. DI water rinse
3. Shutdown
Please select rinse mode and load the selected size of centrifuge tube.
Then click [Start] button to initiate DI rinse procidure.
If you don't need, click [Abort] button.
WARNING: DI rinse must be done after bleach cleaning!
Normal cleaning:
Normal cleaning: 15ml centrifuge tube with 12ml of sodium
Carefull cleaning:
Carefull cleaning: 30ml centrifuge tube with 30ml of sodium
Keep DI water inside sample probe.
Abort
Start
Skip

DI water cleaning starts.
When cleaning with DI water finishes, the following screen appears.

7 Click [OK] to shut down the SH800.

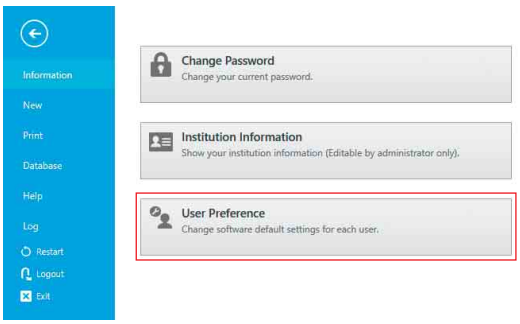


The fluidics system is depressurized and all fluidics lines are sealed.

Specifying User Preferences

Various configuration settings and values can be saved by each user in user preferences. User preferences are loaded automatically each time a user logs in, saving having to reconfigure the instrument each time you want to acquire or analyze data.

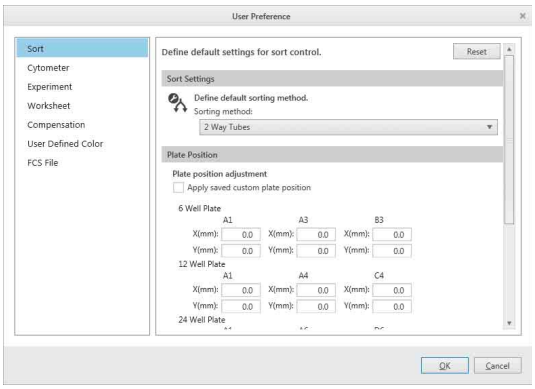
1 Click [Information] on the [File] tab of the ribbon, then click [User Preference].



The [User Preference] dialog appears.

2 Set each item.

The values set here become the default values for each setting.



For details about each item, see "[User Preference] dialog" (page 105).

Exchanging the Sorting Chip

The sorting chip must be changed regularly to ensure an unobstructed flow path.

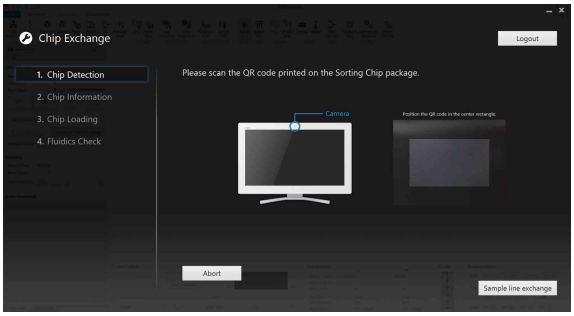
1 Click [Exchange] on the [Cytometer] tab of the ribbon.

A confirmation dialog appears.

2 Click [Yes].

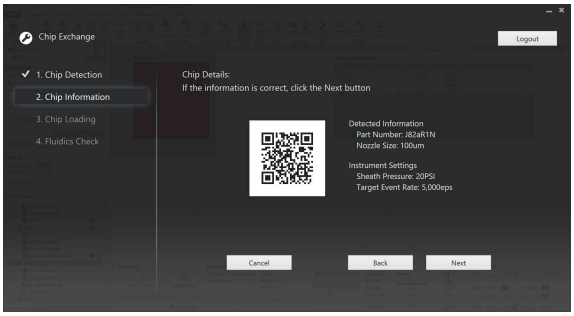
The [Chip Exchange] wizard appears.

3 Scan the QR code on the packaging of the replacement chip.



If the scan is successful, the chip information is displayed.

4 Click [Next].

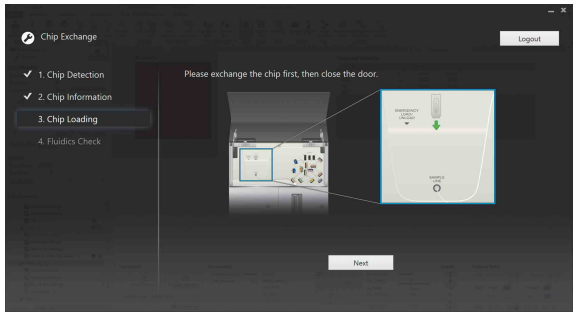


You are prompted to exchange the chip.

5 Open the flip-up door on the front panel, and remove the current sorting chip.

6 Insert the replacement chip in the chip insertion slot, then close the flip-up door.

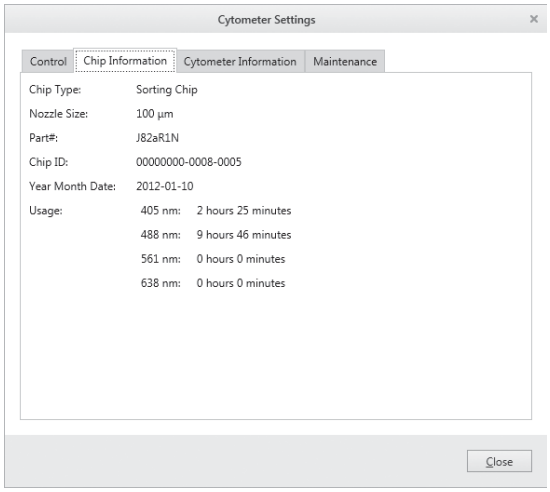
7 Click [Next].



The fluidics check and automatic calibration sequence begins. Follow the on-screen instructions to align the chip and calibrate the instrument.

Notes

- An error message appears if the sorting chip is inserted in the incorrect orientation.
- Do not insert any objects other than Sony sorting and cleaning chips designed for the SH800 into the chip insertion slot.
- The packaging for the loaded sorting chip should be stored in a safe location.
- Replace the sorting chip if it is discolored, dirty, or stained from previous use.
- Do not drop the sorting chip on a hard surface or bend the chip intentionally. If there is a problem with the sorting chip, contact your Sony distributor.
- Always wear gloves and other protective clothing, mask, and goggles, when changing the sorting chip.
- If the sorting chip will not load correctly, run the instrument in maintenance mode as described in *“Running Maintenance Mode” (page 158)* to clean the instrument. If the problem persists, contact your Sony distributor for service.
- The sorting chip must be changed regularly if subjected to prolonged exposure to irradiation by the lasers to prevent burn-in, especially by the 405 nm laser. You can monitor the length of time the chip has been exposed to the light from each laser in the [Chip Information] tab of the [Cytometer Settings] dialog, accessed by clicking [Settings] on the [Cytometer] tab of the ribbon.



Backing Up/Restoring the Database

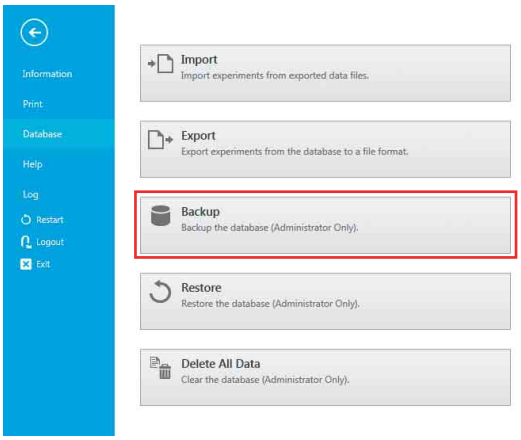
You can back up all data in the SH800 Software database. You can also restore all data from a backed up database, as required.
This operation is available only when logged in using an administrator account.

Tip

- Backup/restore operations are supported on the NTFS-format file system. Other format file systems are not supported.
- Backup operations are performed on external storage only.

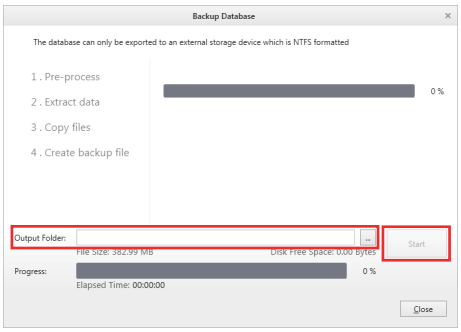
Backing up the Database

1 Click [Database] on the [File] tab of the ribbon, then click [Backup].



The [Backup Database] dialog appears.

2 Click ... (Browse) to specify the backup data destination, then click [Start].



The database backup commences and a progress bar is displayed.
When the database is backed up, a confirmation message appears.

3 Click [OK].

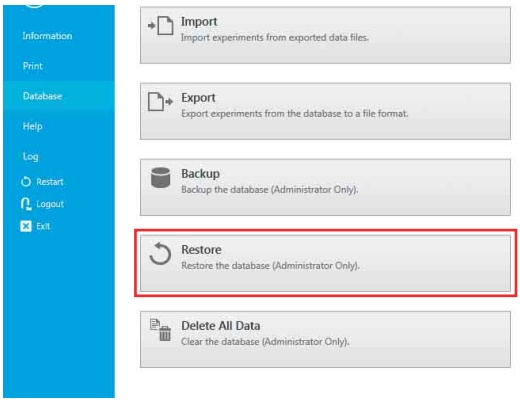
4 Click [Close] to close the [Backup Database] dialog.

Restoring a Database

Tip

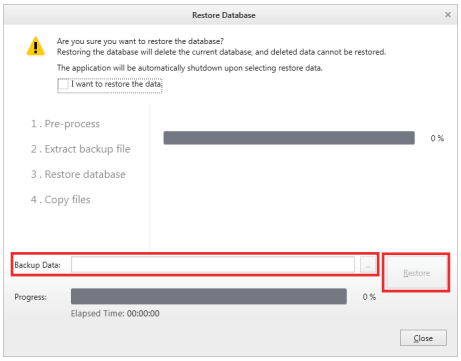
Restoring a database loads the information from a previous database backup, erasing and replacing all information in the current database.

1 Click [Database] on the [File] tab of the ribbon, then click [Restore].



The [Restore Database] dialog appears.

2 Click ... (Browse) to specify the backup data to restore, then click [Restore].


Restore Database
Are you sure you want to restore the database?
Restoring the database will delete the current database, and deleted data cannot be restored.
The application will be automatically shutdown upon selecting restore data.
I want to restore the data.
1 . Pre-process
2 . Extract backup file
3 . Restore database
4 . Copy files
0 %
Backup Data:
Restore
Progress:
0 %
Elapsed Time: 00:00:00
Close

A confirmation message appears.

3 Click [Yes].

SH800 Software launches and the database restore operation starts.
When the database is restored, a confirmation message appears.

4 Click [OK].

5 Click [Close] to close the [Restore Database] dialog.

Chapter 4 Configuring Experiments

This chapter describes how to customize experiments, and how to configure experiment components, detector settings, and fluorescence compensation.

For details creating an experiment quickly, see "Creating an Experiment" (page 47).

Creating a Customized Experiment

You can customize the structure of an experiment created using templates or recent experiments by adding sample groups and sample tubes to the experiment, and configuring the settings of each component.

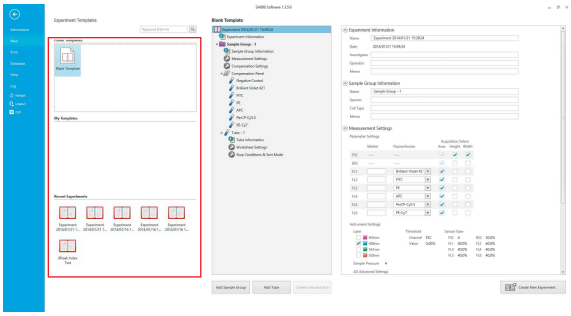
You can also customize an experiment after it has been created. For details, see "Editing an Experiment" (page 74).

1 Click [New] on the [File] tab of the ribbon or click [New] in the [Experiment] group on the [Experiment] tab of the ribbon.

The [Create Experiment] window appears.

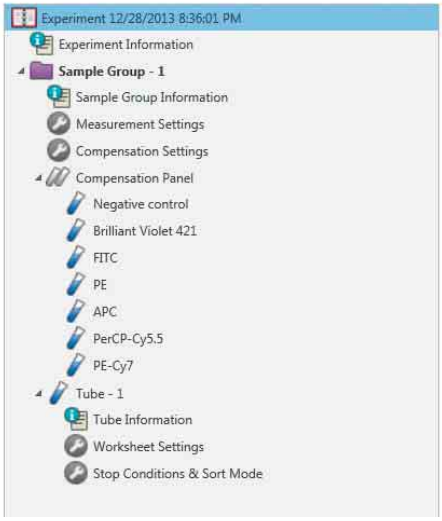
2 Select a template or a recent experiment.

Clicking a template or an experiment on the left displays the structure of the selected experiment on the right side of the window.



3 Modify the structure of the experiment, as desired.

Blank Template

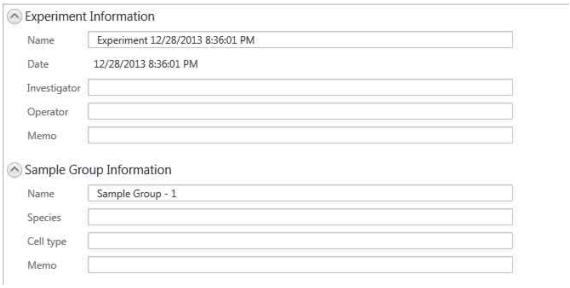


For details about adding/deleting sample groups and sample tubes, see "To add a sample group" (page 71), "To add a sample tube" (page 72), and "To delete a sample group or tube" (page 73).

Tip

Changes made to the settings on the right side of the window are immediately reflected in the experiment structure.

4 Modify the experiment and sample group information, as desired.

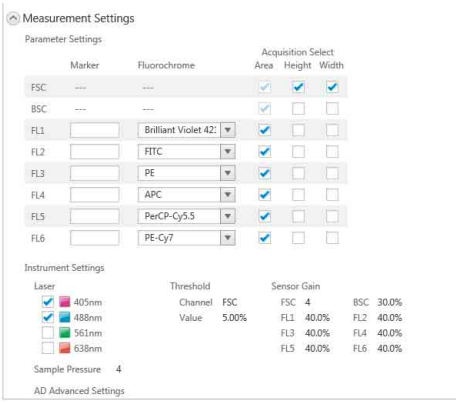


Tip

The name of the experiment is supplied automatically, although it is recommended that you change it to a more appropriate name.

5 Select the detector channel pulse parameters for data acquisition in the [Measurement Settings] pane.

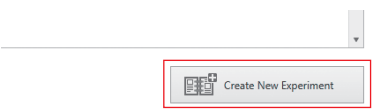
For details, see the [Measurement Settings] dialog description in "Configuring an Experiment" (page 73).



Tips

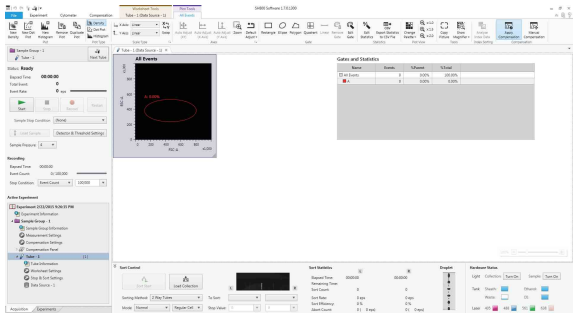
- The fluorochrome and marker names are optional, but recommended.
- Check that the fluorochrome and marker names assigned to detection channels correspond with the laser configuration and optical filter pattern. For details, see *"Fluorochrome Detection Matrix" (page 204)*.

6 When finished building the experiment, click [Create New Experiment] in the bottom right-hand corner of the window.



If "Blank Template" was selected, the [New Experiment Startup Procedure] dialog *(page 48)* appears.

If an existing template or experiment was selected, the main window appears.
The created experiment is automatically made the active experiment, and the first tube is automatically assigned as the active tube.

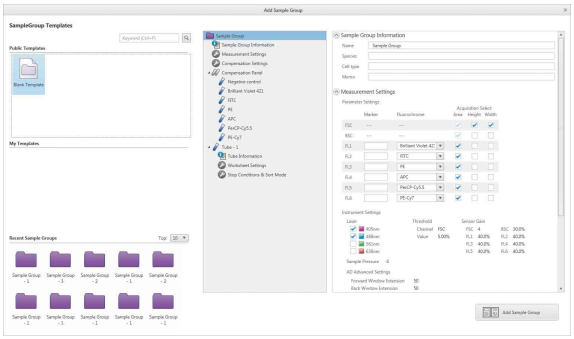


To add a sample group

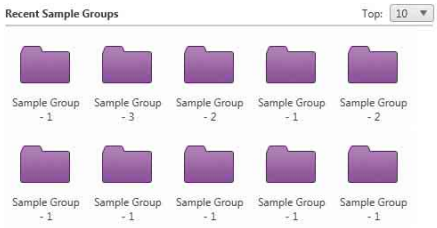
Use the following procedure to add a sample group to an experiment.

1 Click [Add Sample Group] in the [Create Experiment] window.

The [Add Sample Group] dialog appears.



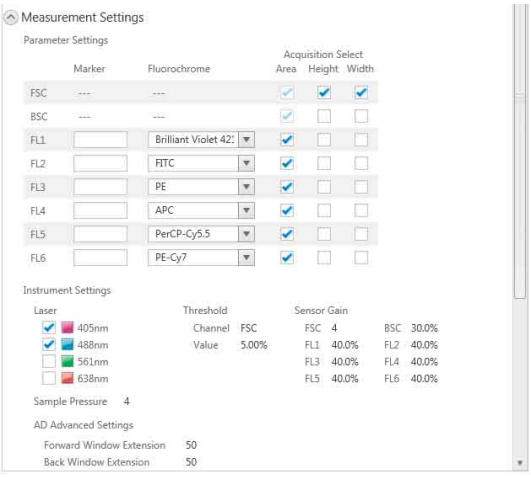
2 Select a sample group template or recent sample group in [Sample Group Templates].



3 Enter a name for the sample group, and modify other settings as required.

The name of the sample group is a required item.

4 Select the detector channel pulse parameters for data acquisition in the [Measurement Settings] pane.



Only control tubes that have a selected detection channel pulse parameter are included in the compensation panel for the sample group in the experiment structure.

Tips

- The fluorochrome and marker names are optional, but recommended.
- Check that the fluorochrome and marker names assigned to detection channels correspond with the laser configuration and optical filter pattern. For details, see *"Fluorochrome Detection Matrix" (page 204)*.

5 Modify the structure of the sample group, as required.

For details, see "To add a sample tube" (page 72) and "To delete a sample group or tube" (page 73).

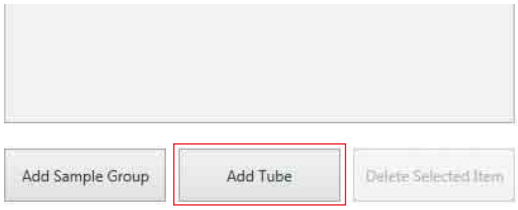
6 When finished, click [Add Sample Group] in the bottom right-hand corner of the window.

The sample group is added to the experiment.

To add a sample tube

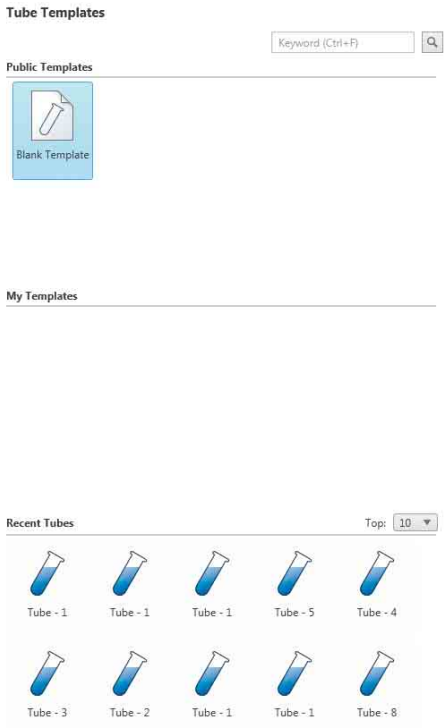
Use the following procedure to add a sample tube to the selected sample group.

1 Click [Add Tube] in the [Create Experiment] window.



The [Add Tube] dialog appears.

2 Select a sample tube template or recent sample tube in [Tube Templates].



3 Enter a name for the sample tube, and modify other settings as required.

The name of the sample tube is a required item.

4 When finished, click [Add Tube] in the bottom right-hand corner of the window.

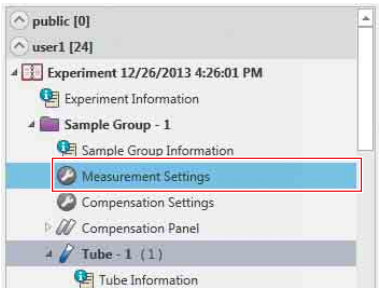
To delete a sample group or tube

Select the sample group or tube you want to delete in the structure tree in the [Create Experiment] window and click [Delete Selected Item].

Configuring an Experiment

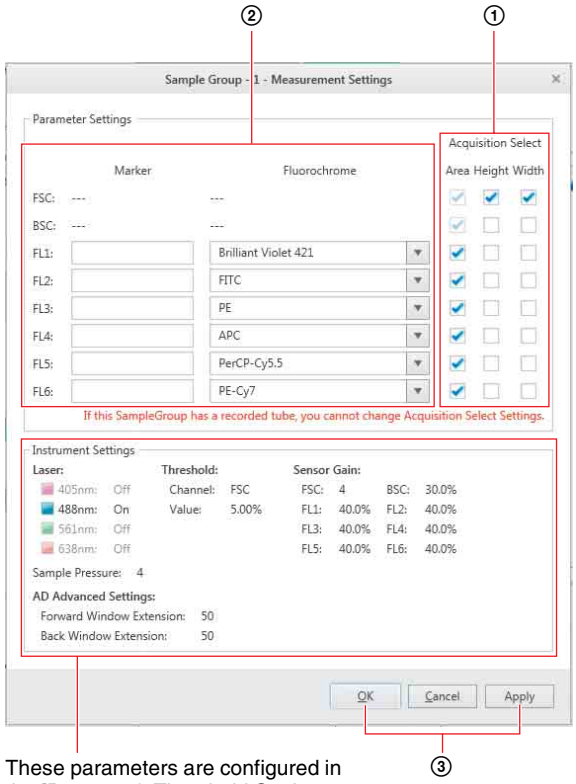
This describes how to configure each component in an experiment.

1 Expand the target experiment in the Experiment Explorer, and double-click [Measurement Settings] in the sample group you want to configure.



The [Measurement Settings] dialog appears.

2 Set each item.



These parameters are configured in the [Detector & Threshold Settings dialog] *(page 79)*.

① Select the pulse parameters (Area, Height, Width) for each detection channel.
The SH800 supports the acquisition of three pulse parameters (area, height, width) in the detector for each of the input channels (FSC, BSC, FL1 to FL6) for each tube in a sample

group. You select the detection channels for which to acquire data by selecting acquisition pulse parameters for those channels. Any combination of pulse parameters can be selected for each detector channel.

Tips

- The Area, Height, and Width pulse parameters are selected by default for the FSC detector channel. Only the Area pulse parameter is selected by default for all other detector channels.
- The Area pulse parameters for the FSC and BSC detector channels are always enabled. Only detector channels with a pulse parameter selection will appear in the compensation panel.

② Specify a marker name and/or fluorochrome name for each fluorescence detector channel.
You can also enter a fluorochrome name directly from the keyboard.
Marker names and fluorochrome names, if specified, appear in the axis labels on plots, and fluorochrome names appear for control tubes in the compensation panel.

Tips

- Forward scatter (FSC) and back scatter (BSC) are measured using the incident laser light, not fluorescent light. Entries for FSC and BSC are not permitted.
- Fluorochrome names and marker names can be modified before, during, or after data acquisition.
- Marker names and fluorochrome names are optional, but recommended. If names are not specified, the fluorescence channel numbers (FL1 to FL6) will be displayed as the axis labels on plots by default, providing limited feedback as to the type of data displayed on plots.

③ Click [Apply] or [OK] to apply the measurement settings.
Clicking [OK] applies the settings and closes the dialog.

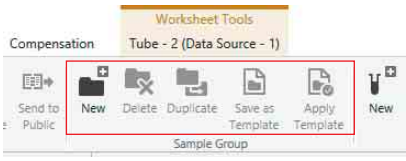
Editing an Experiment

You can modify an existing experiment, as required. You can also edit an experiment using the [Experiment] tab of the ribbon.

For details about tools available on the [Experiment] tab, see "[Experiment] Tab (ribbon)" (page 113).

Editing a Sample Group

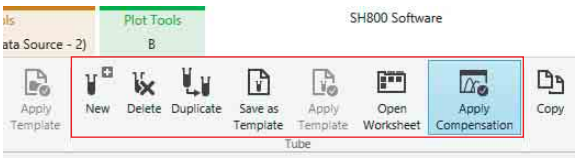
You can add, delete, and perform other edit functions on a sample group in an experiment.
Edit operations are performed using buttons in the [Sample Group] group on the [Experiment] tab of the ribbon.
You can also perform the same operations by right-clicking a sample group in the Experiment Explorer.



For details about each button, see "[Sample Group] group" (page 114) on the [Experiment] tab of the ribbon.

Editing a Sample Tube

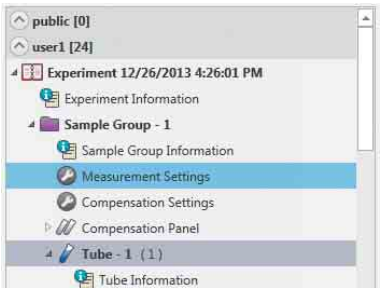
You can add, delete, and perform other edit functions on a sample tube in an experiment.
Edit operations are performed using buttons in [Tube] on the [Experiment] tab of the ribbon.
You can also perform the same operations by right-clicking a sample tube in the Experiment Explorer.



For details about each button, see "[Tube] group" (page 114) on the [Experiment] tab of the ribbon.

Changing Component Settings

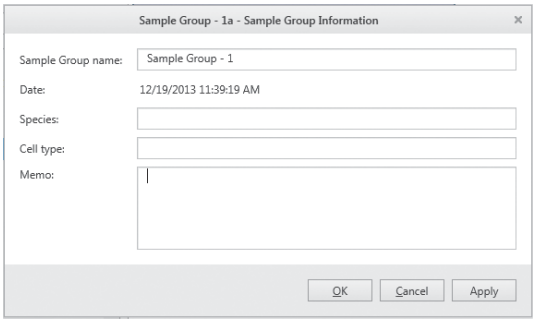
1 Expand the target experiment in the Experiment Explorer, and double-click the component you want to configure.



The corresponding dialog for the selected component appears.

2 Specify each parameter, then click [Apply] or [OK].

Example: If [Sample Group Information] is selected



For details about items displayed in the dialogs, see "Experiment Explorer" (page 133).

For details about [Measurement Settings], see "Configuring an Experiment" (page 73).

Exporting/Importing an Experiment

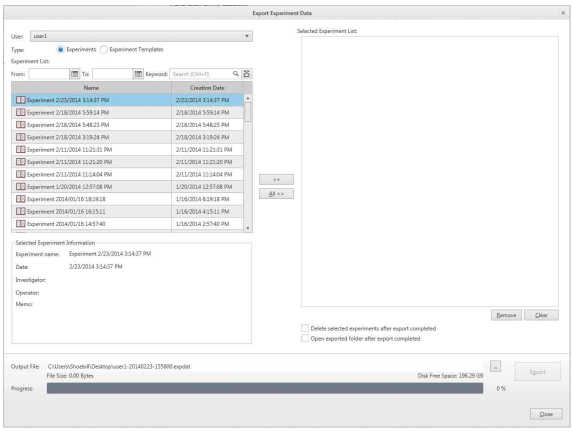
You can import and export experiment data to back up the data, to reopen a previous experiment, or to review the experiment on another SH800 system.

Exporting an Experiment

When data acquisition for an experiment is finished, the data for the experiment can be exported to an external file.

1 Click [Database] on the [File] tab of the ribbon, then click [Export].

The [Export Experiment Data] dialog appears.



2 Select the owner of the data in [User], and select to export experiments or templates using the [Type] radio buttons.

3 Select an experiment you want to export in [Experiment List], and click [>>] to move it to [Selected Experiment List].

Clicking [All >>] moves all the experiments to [Selected Experiment List].

Tips

- To remove the exported experiments from the Experiment Explorer after the export is finished, place a check mark in [Delete selected experiments after export completed].
- To open the export destination folder after the export is finished, place a check mark in [Open exported folder after export completed].

4 Click [...] (Browse) to specify the destination folder in which the experiment data is to be exported.

5 Click [Export].

The export status is displayed in the progress bar. A confirmation dialog appears when exporting is completed.

6 Click [OK].

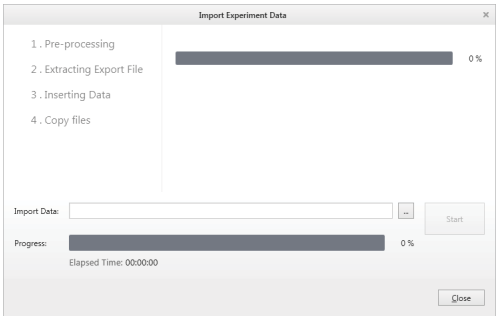
7 Click [Close] to close the dialog.

Importing an Experiment

You can import previously exported experiment data into the database.

1 Click [Database] on the [File] tab of the ribbon, then click [Import].

The [Import Experiment Data] dialog appears.



2 Click ... (Browse) to specify the folder where the experiment data you want to import (.expdat file) is stored.

3 Click [Start].

The import status is displayed in the progress bar. A confirmation dialog appears when importing is completed.

4 Click [OK].

5 Click [Close] to close the dialog.

Sharing Experiments

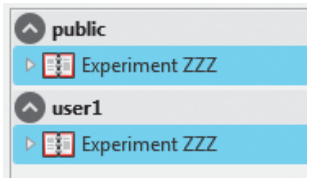
You can share experiments with other users.
Shared experiments allow multiple users to work with the experiment data on worksheets.

To share an experiment

1 Select the experiment you want to share in the Experiment Explorer, and click [Send to Public] on the [Experiment] tab of the ribbon.

Alternatively, you can right-click an experiment and select [Send to Public] from the context menu.

The shared experiment is duplicated in the [public] node in the Experiment Explorer.



Tip

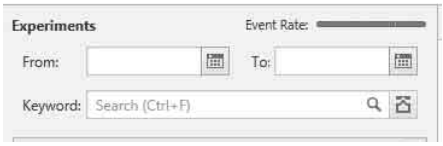
Other users can access the shared experiment under the [public] node. After an experiment is shared, the shared experiment and the original private experiment exist as independent experiments, with no interaction between the two.

Searching Experiments

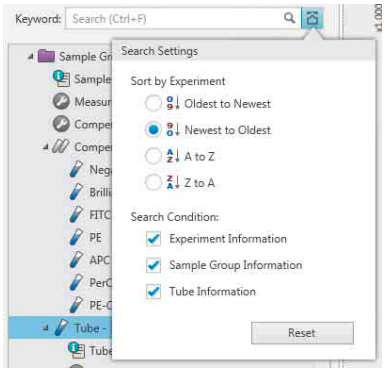
You can search experiments by date and keyword.

1 Enter the search criteria in the Experiment Explorer of the [Experiments] tab on the left side of the main window.

Clicking displays a calendar for specifying a date.



2 Click to set optional addition search options.



[Oldest to Newest]: Displays the search results in chronological order from oldest to newest.
[Newest to Oldest]: Displays the search results in chronological order from newest to oldest.
[A to Z]: Displays the search results in ascending alphabetical order.
[Z to A]: Displays the search results in descending alphabetical order.
[Search condition]: Selects the experiment components to search for the entered text keyword.
[Reset]: Resets the search options to their defaults.

Experiments matching the search criteria are displayed in the Experiment Explorer.

Saving Tube Data as FCS Files

You can export data recorded for tubes in flow cytometry industry standard FCS 3.0 or FCS 3.1 format for use in third-party applications.

Note

SH800 Software does not support the importing of FCS format files.

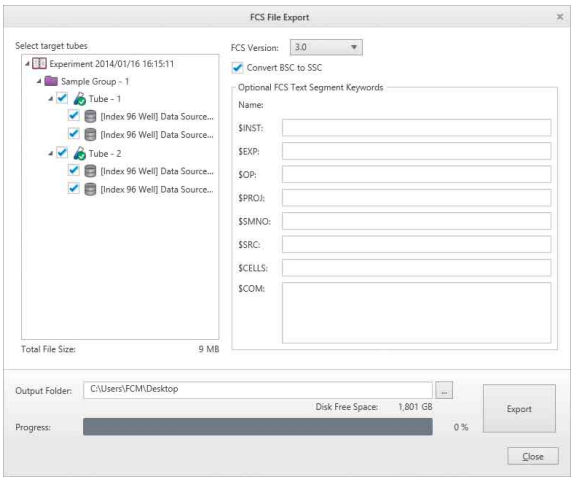
1 Select the target tube in the Experiment Explorer on the [Experiments] tab on the left of the main screen.

2 Click [Export to FCS file] in the [Export] group on the [Experiment] tab of the ribbon.

The [FCS File Export] dialog appears.

3 Place check marks in [Select target tubes] for tubes whose data is to be exported, specify the FCS format and export destination folder, then click [Export].

You can also select the required data source if there is more than one data source for a single tube.



The tube data is exported.
The name of the exported file is the same as the name of the tube, with an ".fcs" filename extension.

Tips

- [Convert BSC to SSC]: Selects whether to change the sensor name for data recorded by the "BSC" (back scatter) detector to "SSC" (side scatter) when exporting FCS files.

- The required FCS text segment keywords are exported automatically. The following optional FCS text segment keywords can also be specified.

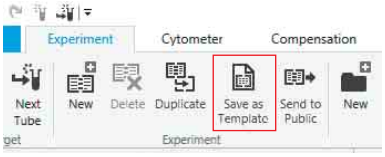
Keyword	Description
$INST	Institution at which data was acquired
$EXP	Name of investigator initiating the experiment
$OP	Name of flow cytometer operator
$PROJ	Name of the experiment project
$SMNO	Specimen (e.g., tube) label
$SRC	Source of the specimen (patient name, cell type)
$CELLS	Description of objects measured
$COM	Comment

Saving an Experiment as a Template

You can save an experiment, sample group, or sample tube as a shared template (Public Templates) or a private template (My Templates.)

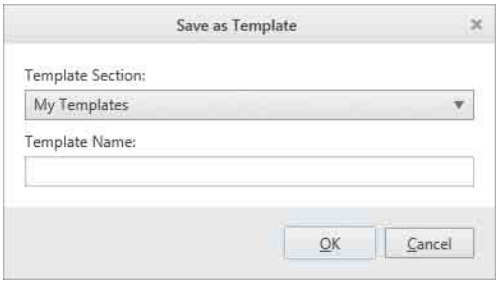
1 Select the target experiment in the Experiment Explorer on the [Experiments] tab on the left of the main screen.

2 Click [Save as Template] in the [Experiment] group on the [Experiment] tab of the ribbon.



The [Save as Template] dialog appears.

3 Select the template type, enter a name, and then click [OK].



The experiment is saved as a template.

Configuring Detector Settings

The following instrument settings must be optimized in an experiment to acquire accurate data.

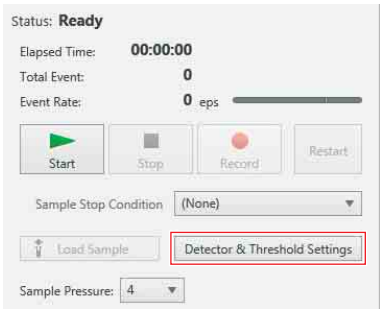
- Trigger threshold channel
- Trigger threshold value
- Sensor gain of the forward scatter, back scatter, and fluorescence detectors

The instrument settings are saved with the sample group in an experiment. Duplicating an experiment or creating a new experiment using templates will load the saved instrument settings into the new experiment. Running an experiment using the same instrument settings as a previous experiment enables direct comparisons to be made between the results obtained, even if the experiments were conducted at different times.

You can also configure many of the same parameters using the [Compensation Wizard] and on the [Control] tab of the [Cytometer Settings] dialog.

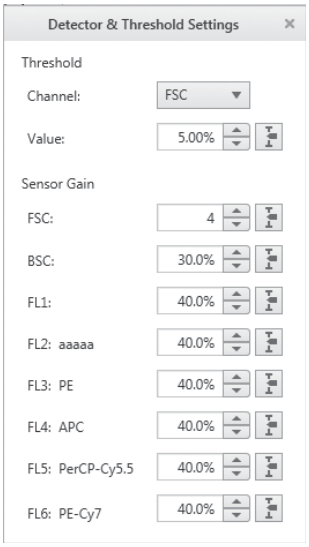
For details, see "Performing Fluorescence Compensation" (page 61) and "[Cytometer Settings] dialog" (page 121).

1 Click [Detector & Threshold Settings] in the [Acquisition] tab of the Experiment/Acquisition control pane.



The [Detector & Threshold Settings] dialog appears.

2 Set each item.



[Threshold]

Specifies the threshold for event detection.

[Channel]: Selects the channel (FSC, BSC, FL1 to FL6) used to trigger the detection of events. The default trigger channel is FSC.

[Value]: Sets the threshold value for the trigger channel as a percentage of the output signal of the detector for the trigger channel. The default value is 5.00%.

[Sensor Gain]

Specifies the sensor gain of the detectors.

[FSC]: Specifies the gain for the forward-scatter detector. The gain setting has a 16-step range.

[BSC]: Specifies the gain of the back scatter photomultiplier tube (PMT) detector as a percentage of the output signal of the detector. The default value is 30.0%.

[FL1] to [FL6]: Specifies the gain of the corresponding fluorescence channel photomultiplier tube (PMT) detector as a percentage of the output signal of the detector. The default value is 40.0%.

Tips

- Only the gain of fluorescence channels that have pulse parameters selected for acquisition can be adjusted. The gain settings of other channels are disabled.
- If the PMT gain is too high, the detector output circuit may become saturated, providing incorrect signal output. In this case, reduce the gain and then readjust the instrument settings to find the optimum operating point.

3 Click [X] to close the dialog.

The settings are saved.

Note

The gain parameter values directly affect the fluorescence compensation calculation of the spillover matrix. Do not change the gain of the channel detectors after or while recording the control tubes in a compensation panel. The fluorescence compensation process is valid only for the instrument settings in place when all control tubes in a compensation panel are recorded. If the gain of a detector for any channel is changed for any reason after recording the control tubes, the fluorescence compensation will no longer be valid and the control tubes will need to be loaded and recorded again.

For details about recording control tubes in a compensation panel, see "Performing Fluorescence Compensation" (page 61).

Tip

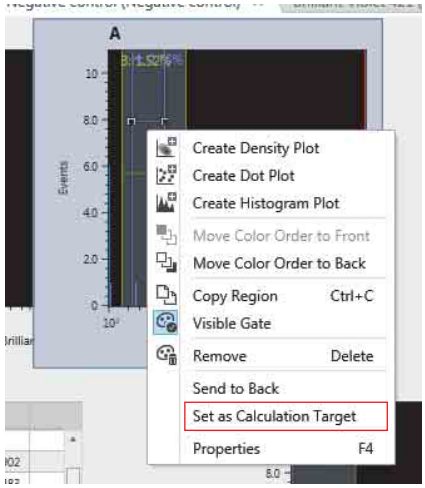
There are various other methods that can be used to optimize the detector gain settings for an experiment. For example, a more rigorous approach to detector gain optimization might be to measure the coefficient of variation (CV) for positive controls at various detector gain levels to determine the minimum gain levels for which the CV plateaus off in each detection channel sensor. A detector gain set at this minimum gain level will provide the optimal signal-to-noise ratio required for maximum sensitivity and maximum separation between negative and positive peaks.

Adjusting Fluorescence Compensation

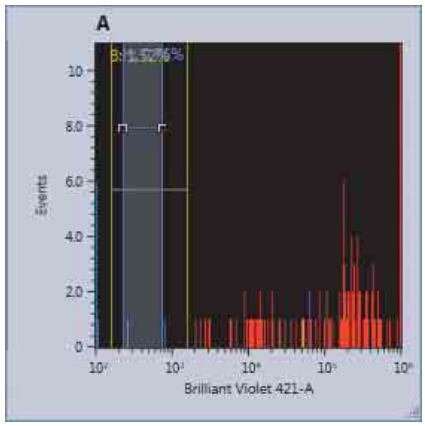
Using a Target Gate in the Gate Hierarchy

You can specify the data for a gate within the gate hierarchy to be used as the negative sample data or a singly-stained positive sample data by setting the gate as the target for calculating the compensation matrix.

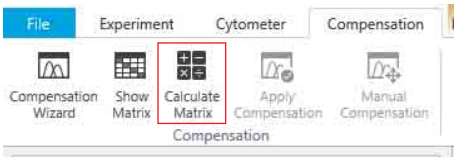
1 Right-click a gate on a plot for a control tube in the compensation panel to set the gate as the calculation target for the compensation matrix, and select [Set As Calculation Target] from the context menu.



The selected gate is highlighted.

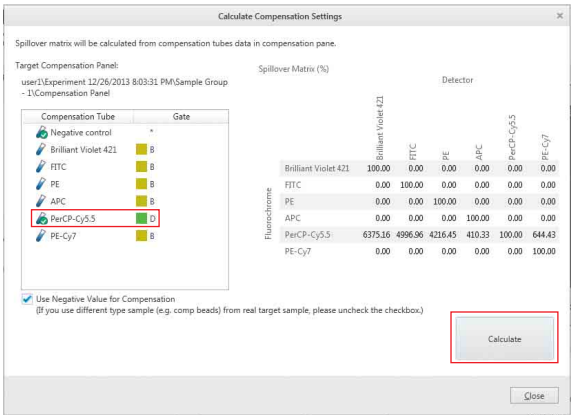


2 Click [Calculate Matrix] on the [Compensation] tab of the ribbon.



The [Calculate Compensation Settings] dialog appears.

3 Check that the specified gate is the target of the calculation, and click [Calculate] to calculate the spillover matrix.



4 Click [Close] to close the dialog.

Adjusting Fluorescence Compensation Manually

You can modify the compensation settings after using the [Compensation Wizard] or you can enter fluorescence compensation values manually to edit the spillover matrix directly.

Tips

- You can save the current fluorescence compensation settings using the [Compensation Settings] dialog.

  For details about displaying the [Compensation Settings] dialog, see "To edit the spillover matrix directly" (page 82).

- Adjusting the position of events shown on a plot also adjusts the position of all events on other plots. Accordingly, you should add plots for all fluorescence parameters to the worksheet before adjusting compensation manually in order to monitor the affect of adjusting the compensation on each plot.

To adjust compensation graphically on plots

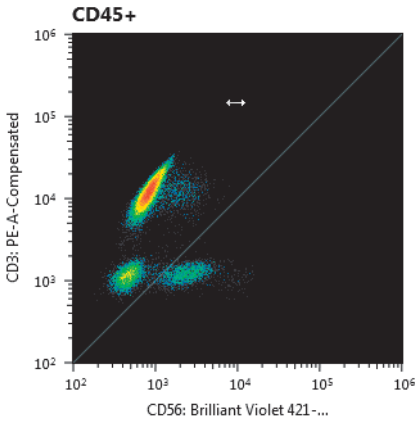
You can adjust fluorescence compensation graphically on the worksheet using a mouse.

1 Display the worksheet for the tube whose compensation you want to modify.

2 Enable [Apply Compensation] on the [Compensation] tab of the ribbon.

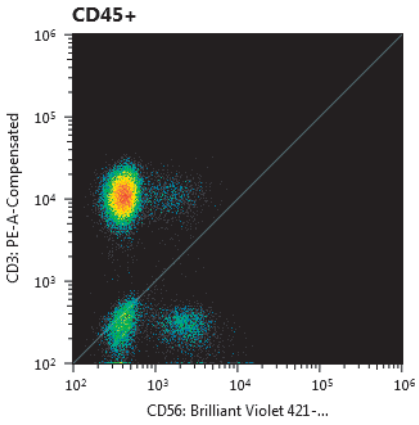
3 Click [Manual Compensation] on the [Compensation] tab of the ribbon to activate manual compensation mode.

A diagonal line appears on plots that have fluorescence parameters on the X and Y axes.



4 Place the mouse cursor over the plot, and drag the plot to change compensation for the target parameter.

The mouse cursor changes to a horizontal double-ended arrow cursor in the left half of the plot, and a vertical cursor in the right half of the plot.
The displayed events move on the plot to reflect the changed compensation.



5 When finished, click [Manual Compensation] again to deactivate manual compensation mode.

You can also deactivate manual compensation mode by selecting another worksheet to display.

Tips

- Adjusting the position of events shown on a plot also adjusts the position of all events on other plots. Accordingly, you should add plots for all fluorescence parameters to the worksheet before adjusting compensation manually in order to monitor the affect of adjusting the compensation on each plot.
- The actual compensation values in the spillover matrix can be monitored simultaneously in the [Compensation Settings] dialog. It is recommended that you save the compensation settings from the [Compensation Settings] dialog before adjusting compensation manually. You can also click [Reset] in the [Compensation Settings] dialog to reset the compensation settings.

To edit the spillover matrix directly

You can adjust the fluorescence compensation manually in the [Compensation Settings] dialog. You can adjust the values using visual feedback alone, or enter spillover matrix values directly to reproduce the compensation settings of another experiment.

Note

Editing the spillover matrix directly to adjust compensation is inherently difficult. Exercise care when editing the spillover matrix directly.

1 Display the worksheet for the tube whose compensation you want to modify.

2 Click [Apply Compensation] on the [Experiment] tab of the ribbon.

The fluorescence compensation obtained using the [Compensation Wizard] is applied to the plots on the worksheet.

3 Display the [Compensation Settings] dialog by doing one of the following.

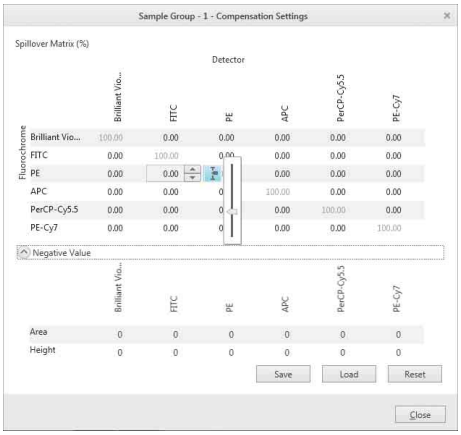
- Select [Compensation Settings] in the Experiment Explorer and click [Show Settings] on the [Experiment] tab of the ribbon.
- Double-click [Compensation Settings] in the Experiment Explorer.
- Select any component within the sample group and click [Show Matrix] on the [Compensation] tab of the ribbon.

The [Compensation Settings] dialog appears.

4 Click a cell in the matrix to select its value.

You can adjust a value using one of the following methods.

- Turn the mouse scroll wheel button.
- Enter a value directly in the spillover matrix.
- Click the spin boxes.
- Drag the slider.



The plots on the worksheet are updated in real-time.

Tips

- The number of fluorochromes in the experiment determines the dimension of the spillover matrix. Spillover elements can be modified, but they cannot be added nor deleted.
- To save the current compensation settings, click [Save]. To load compensation settings for the sample group, click [Load].
- To reset the compensation settings, click [Reset].

5 Click [Close] to close the dialog.

Analysis

Chapter 5

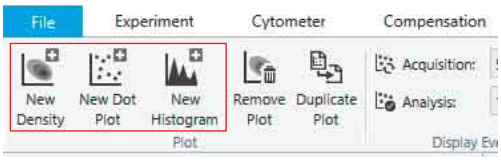
Data is analyzed using various tools available on the worksheet.
This chapter describes gate settings used for analysis of the acquired data and for sorting, generating reports of data analysis, and related tasks.

For details about content displayed on the main window, see *"Main Window" (page 113)*.

Adding a Plot

You can add plots to the worksheet.

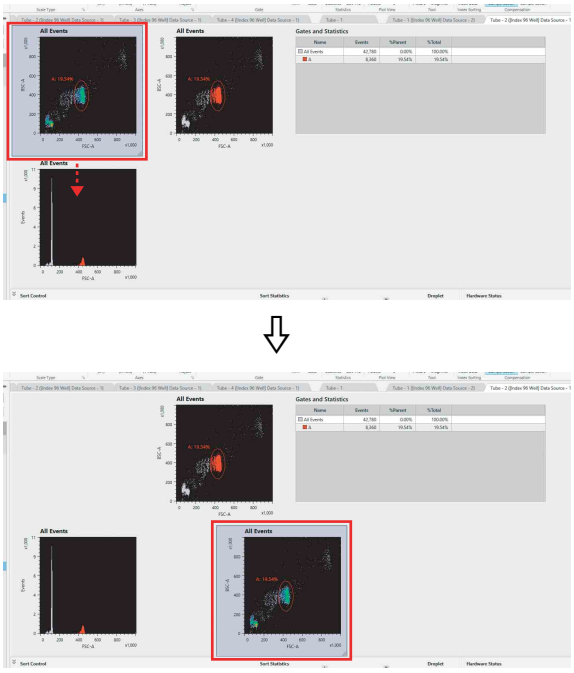
1 Click a button for a new plot of the desired plot type to be added in the [Plot] group on the [Worksheet Tools] tab or the [Plot Tools] tab of the ribbon.



The plot is added to the worksheet. The new plot created represents the [All Events] population.

2 Change the layout of plots, as required.

You can change the position of plots by selecting the target plot and dragging it to a new location.



Edit the plots, add gates, and perform other actions on plots, as required.

See "Editing Plots" (page 84) and "Adding Gates" (page 88).

Editing Plots

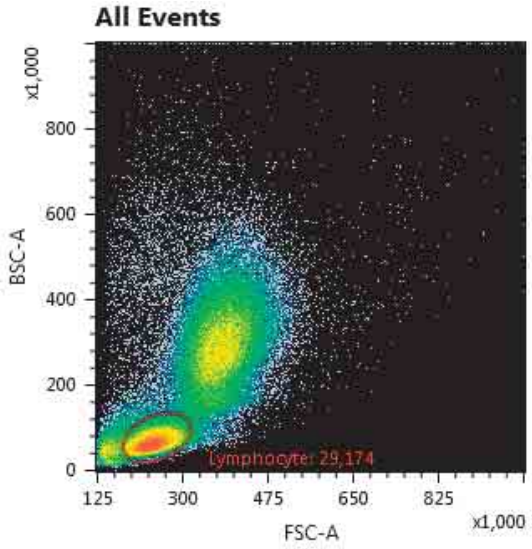
SH800 Software displays data on the following types of plots. You can change the plot type, change the axis scale, and modify other aspects of plots.

For details about the buttons on the [Plot Tools] tab of the ribbon, see "[Plot Tools] Tab (ribbon)" (page 129).

For details about adding gates to a plot, see "Adding Gates" (page 88).

Density plots

Density plots display a frequency distribution of fluorescence intensity for two acquisition parameters (bivariate distribution).
Density plots use color to show the distribution of the number of detected events having the same value. High-density regions are shown in red, low-density regions are shown in violet, and regions of intermediate density are shown using the colors of the visible spectrum in between. Density plots are most useful for displaying populations where the number of detected events is relatively large.

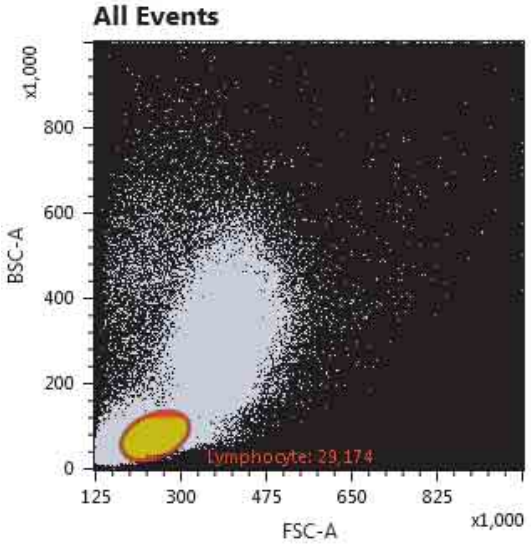


Dot plots

Dot plots display a frequency distribution of fluorescence intensity for two acquisition parameters (bivariate distribution).
Dot plots are most useful for displaying populations where the number of detected events is relatively small. Events on a dot plot are colored based on their classification (gate and population).

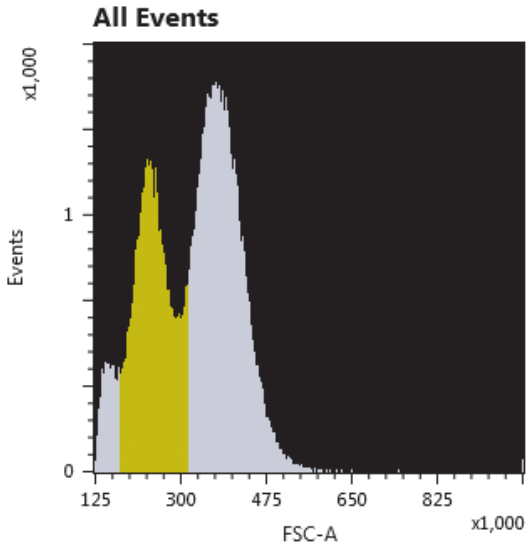
Tip

As the number of events on a dot plot increases, the plot may become saturated making it difficult to discern patterns. In this case, reduce the number of events displayed or use a density plot to view the data.



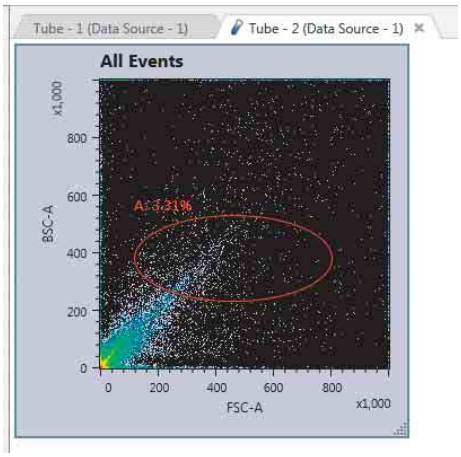
Histogram plots

Histogram plots display a frequency distribution of fluorescence intensity for a single acquisition parameter (univariate distribution).
Histogram plots are effective for identifying the peaks and troughs in measurement data.



Changing Plot Type

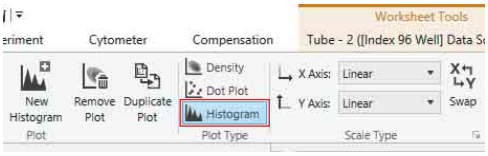
1 Select a plot whose type you want to change on the worksheet.



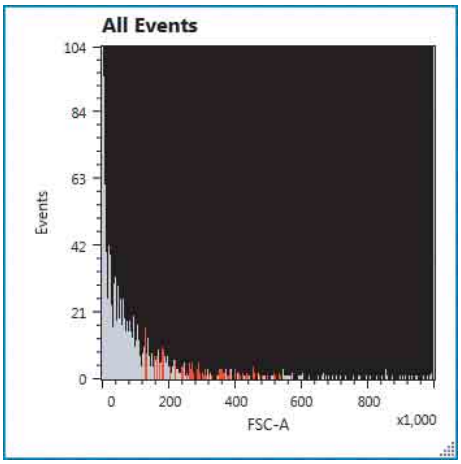
2 Click the target plot type in the [Plot Type] group on the [Plot Tools] tab of the ribbon.

You can also right-click a plot and select a plot type from the [Plot Type] submenu of the context menu.

For example, the diagram below shows a density plot being changed to a histogram plot.



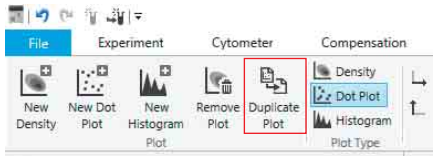
The type of the selected plot is changed.



Duplicating a Plot

1 Select a plot to be duplicated on the worksheet, then click [Duplicate Plot] in the [Edit] group on the [Plot Tools] tab of the ribbon.

You can perform the same operation from the context menu by right-clicking the plot.



The selected plot is duplicated.

2 Edit the duplicated plot, as required, using the buttons on the [Plot Tools] tab of the ribbon.

You can perform the same operation from the context menu by right-clicking the plot.

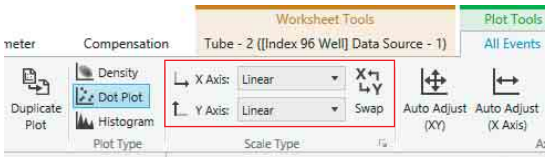
For details about each button, see "[Plot Tools] Tab (ribbon)" (page 129).

Removing a Plot

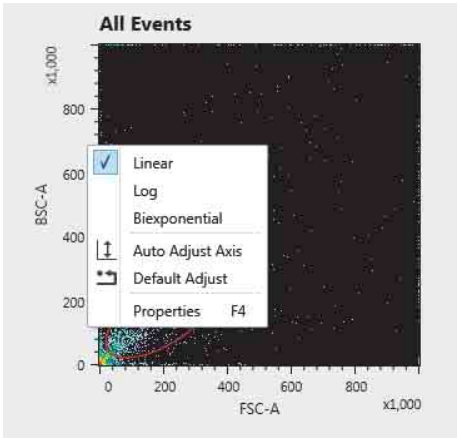
Select a plot to be deleted on the worksheet, then click [Remove Plot] in the [Plot] group on the [Plot Tools] tab of the ribbon.

Changing Axis Scales

Select a plot on the worksheet, then click the desired scale for each axis in the [Scale Type] group on the [Plot Tools] tab of the ribbon.



You can also change the scale by right-clicking the plot and selecting from the context menu.



For details about each item, see "[Scale Type] group" (page 129).

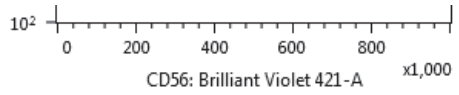
You can select the following scale types.

Tip

The axis scale type is applied only to the display of data on plots. Internally, all data is linear.

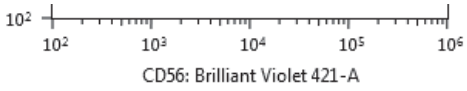
Linear

Performs a linear transformation between the measurement values and the channel numbers (bins) used to measure data. Linear scales are typically used to display forward scatter and back scatter parameters on plots.



Log (logarithmic)

Logarithmic (log) scales are useful for displaying cells that exhibit fluorescence with intensities across a wide dynamic range. A logarithmic scale helps to make individual peaks easier to visualize. However, log scales cannot adequately represent data with a low mean and high variance.



Biexponential

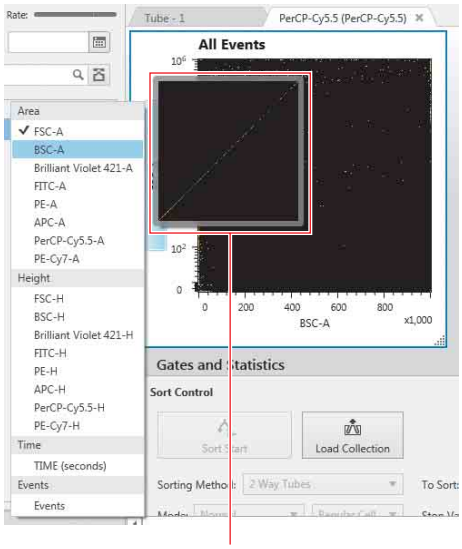
Biexponential scales combine a linear scale at the lower end of the axis (around zero) and a logarithmic scale at the higher end of the axis, with an algorithmic transition between the two scales. A biexponential scale allows events close to or below zero to be plotted so that this population of events can be visualized, making it easier to include or exclude populations when setting up gates.

A common problem occurs when displaying fluorescence data on a logarithmic scale after fluorescence compensation has been applied to a tube. The data transformation used during fluorescence compensation inherently introduces further variance into the measurement data due to variations in detector sensitivity, background noise, and other factors. The result is that some data values can become close to zero or even negative. When values close to zero are plotted on a log scale, they are wedged up against the axis making them very difficult to discern. In addition, values below zero cannot be displayed at all. This can lead to these events, which are real events, being excluded from gates or other analysis. It can also lead to inaccurate fluorescence compensation when attempting to adjust compensation manually using visual cues.

In these cases, a biexponential scale can be used to avoid some of the pitfalls of logarithmic scales, while maintaining the overall utility of logarithmic scales.

Changing the Axis Parameters

Click the axis title and select the desired parameter from the popup menu.

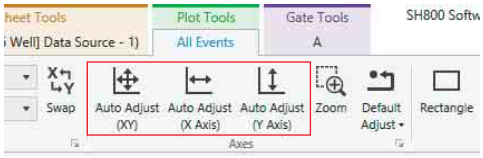


A preview of the plot appears as the mouse hovers over each

Adjusting Scale Ranges

To adjust a scale range automatically

Select a plot, then click one of the automatic scale adjustment buttons in the [Axes] group on the [Plot Tools] tab of the ribbon.



[Auto Adjust (XY)]: Automatically adjusts the scale of the X axis and Y axis.

[Auto Adjust (X Axis)]: Automatically adjusts the scale of the X axis only.

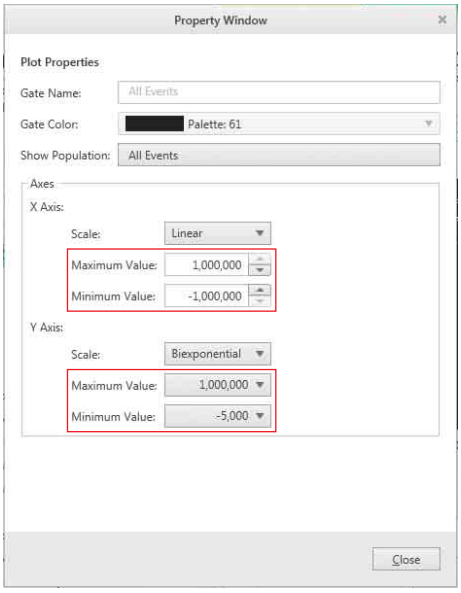
[Auto Adjust (Y Axis)]: Automatically adjusts the scale of the Y axis only.

To specify scale ranges manually

1 Select a plot, then click the dialog launcher in the [Axes] group on the [Plot Tools] tab of the ribbon.

The [Property Window] dialog appears.

2 Enter values for the minimum and maximum values for the X axis and Y axis in [Axes].

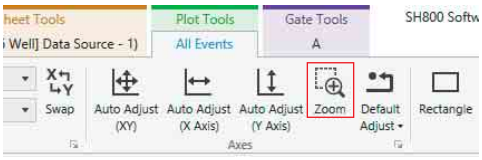


3 Click [Close] to close the dialog.

To zoom in using a mouse

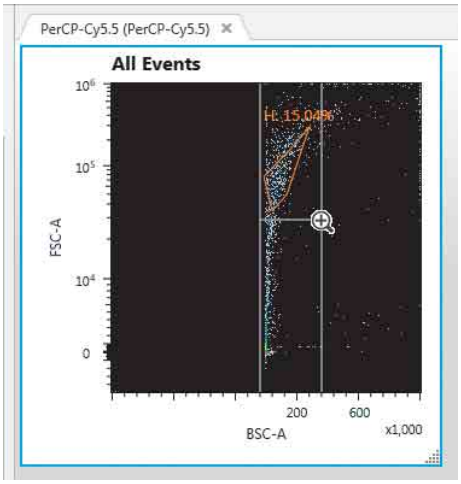
You can enlarge a portion of the plot you want to display using the mouse.

1 Select a plot, then click [Zoom] in the [Axes] group on the [Plot Tools] tab of the ribbon.

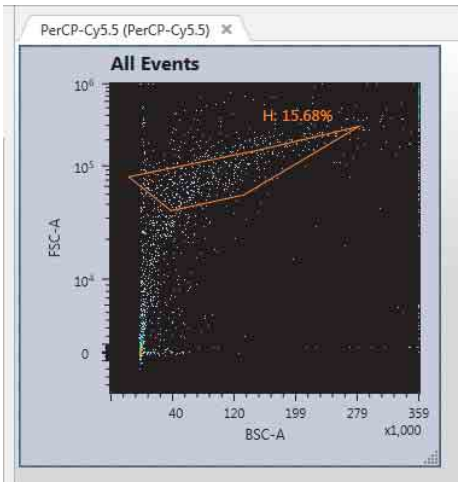


The mouse cursor changes to when the mouse is placed over the selected plot.

2 Drag a selection rectangle over the portion of the plot to display then release the mouse.



The selected range is scaled to fill the plot area.



Tip

Gates change shape on plots to track the scale type and zoom factor, but may not be always reproduced accurately. In particular, calculation of polygon gates and ellipse gates are prone to error when a scale is changed from one type to another.

Adding Gates

You can isolate populations of events by drawing gates around the events on plots. Gates of different types (Rectangle, Ellipse, Polygon, Quadrant, Linear) are available which can be drawn to match the distribution of events. Adding a gate allows you to display a new plot representing the population of events that fall within the gate.
Gates are automatically added to the gate hierarchy in the [Gates and Statistics] table when they are added to plots.

You can also create Boolean gates which are defined by a logical combination of existing gates using Boolean logic. Boolean gates are defined not by drawing gates on plots, but by specifying an equation for the gate. The populations defined by Boolean gates can be displayed on plots, just like populations from physical gates. All statistics functions available for physical gates are also available for Boolean gates.

Physical gates and Boolean gates can be selected as the sorting criteria when sorting.

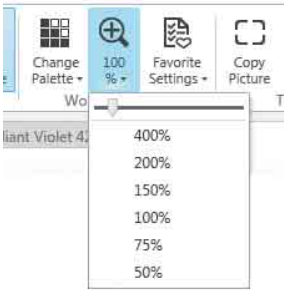
For details, see "Sorting" (page 93).

Adding a Physical Gate

You add a physical gate directly to plots.

1 Select a plot on which to add a gate.

Change the plot zoom, as required, in the [Worksheet View] group on the [Worksheet Tools] tab of the ribbon to make it easier to draw the gate.



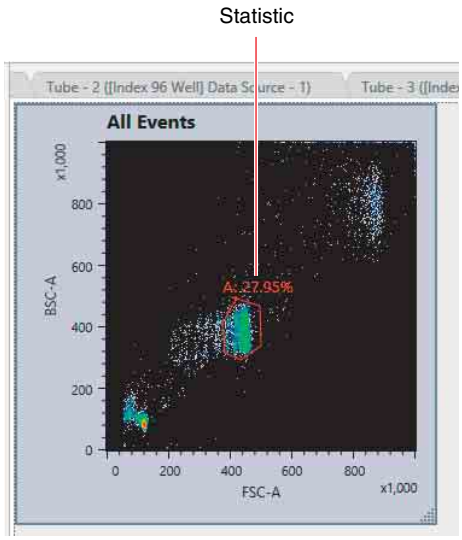
2 Click the desired gate type button in the [Gate] group on the [Plot Tools] tab of the ribbon, and draw a gate on the plot.

Alternatively, right-click the plot, then select [Create gate] and the desired shape of gate from the context menu.

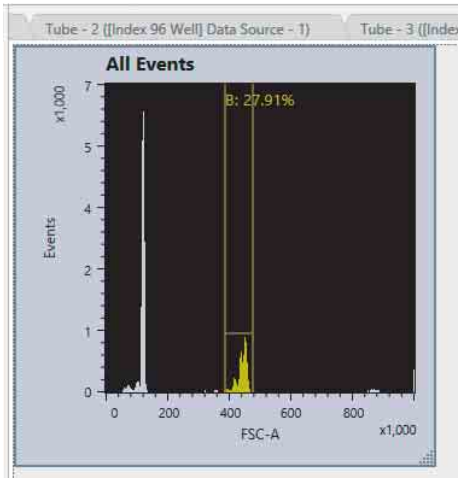
[Rectangle]: Adds a rectangle gate.
[Ellipse]: Adds an ellipse gate.
[Polygon]: Adds a polygon gate.
[Quadrant]: Adds a quadrant gate. A quadrant gate is created on a plot by clicking the mouse at the intersection point of the four quadrants.
The four quadrants ("<gate_name>-Q1" to "<gate_name>-Q4") are added to the plot.
Drag the intersection point to change the size of the quadrants.
[Linear]: Adds a linear gate. Linear gates can be added to histogram plots only.

The gate is added to the plot, together with a single statistic for the gate.

Example: Polygon gate

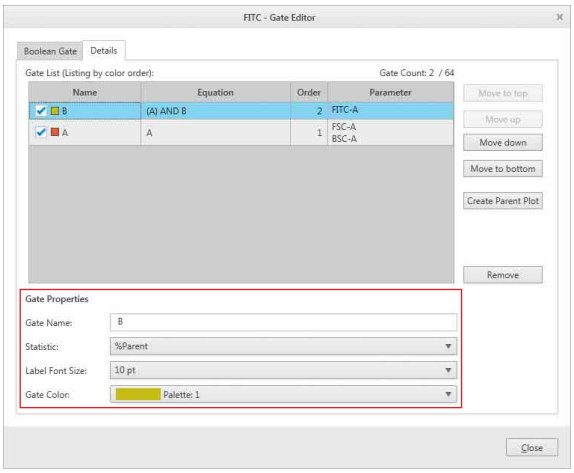


Example: Linear gate



3 Edit the name of the gate, the displayed statistic, color, and other preferences, as required.

Select the gate, click [Edit Gate] in the [Gate] group on the [Plot Tools] tab of the ribbon to display the [Gate Editor] dialog, and then select the [Details] tab.

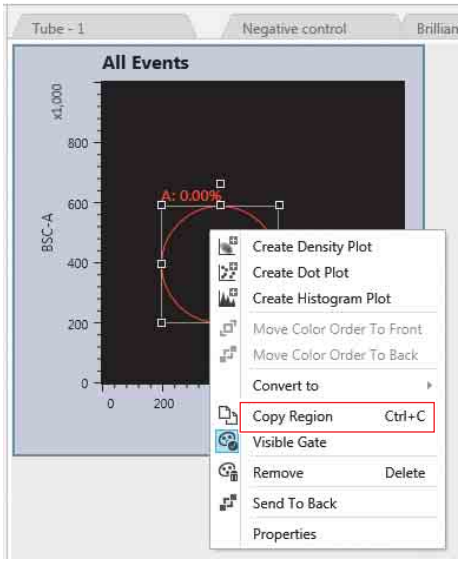


For details about each item, see "[Gate Editor] dialog" (page 127).

Copying a Physical Gate

You can copy a gate drawn on a plot to another plot.

1 Right-click a gate to copy, then select [Copy Region] from the context menu.



2 Paste the gate on the target plot.

Tip

You can also right-click a gate in the [Gates and Statistics] table to copy a gate within the gate hierarchy using commands in the context menu.

Adding a Boolean gate to the Gate Hierarchy

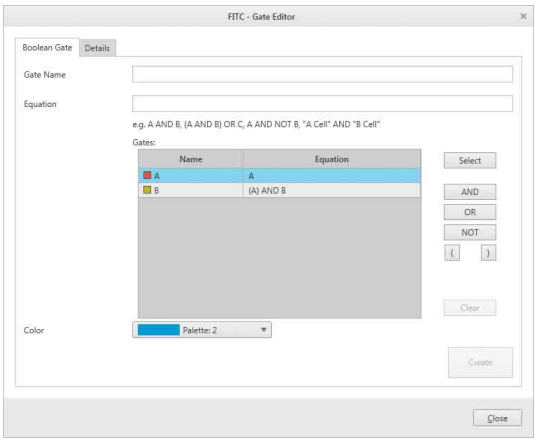
1 Select a plot to display the [Plot Tools] tab.

2 Click [Edit Gate] in the [Gate] group on the [Plot Tools] tab of the ribbon.

The [Gate Editor] dialog appears.

3 Enter the equation for the Boolean gate on the [Boolean Gate] tab.

Enter an equation as follows.



① Click the [Equation] text box to place the cursor in the box.

② Select a gate in the [Gates] list and click [Select], or double-click the name of the gate.
The gate is added to the equation.

③ Click [AND], [OR], [NOT], [(], or [)], as required.
The logic operator is added to the equation.
You can also enter the equation directly from the keyboard.

④ Repeat steps ② and ③ to build the equation for the gate.

Tip

The equations for all gates can be viewed on the [Details] tab.

⑤ Change the name of the Boolean gate, as required.
Click in the [Gate Name] text box, and enter a new name.
The names of gates must be unique.

⑥ Select a color for the Boolean gate from the [Color] drop-down menu.

⑦ Click [Create].
If the equation specified for the gate is verified as a valid definition for a gate, the gate is added to the bottom of the gate hierarchy in the [Gates and Statistics] table.

⑧ Click [Close] to close the dialog.

4 Double-click the Boolean gate in the gate hierarchy.

A default FSC v. BSC density plot with linear X axis and Y axis is added to the worksheet.

5 Set the axis labels, scale types, and other properties for the new plot.

For details, see "Editing Plots" (page 84).

Editing Gates

You can change the shape of and settings for gates on plots.

To change the shape of a gate

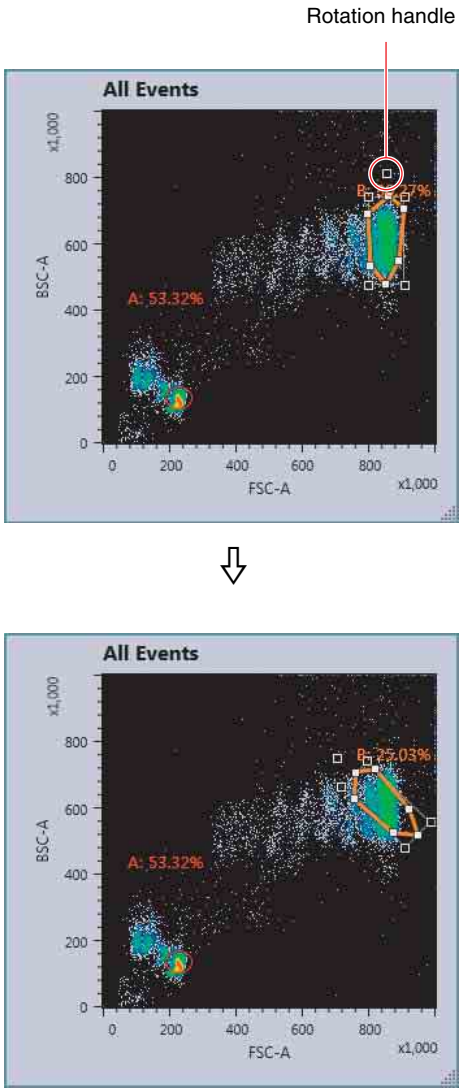
Select the gate and drag the drawing handles to change the shape.
For quadrant gates, drag the intersection point of the quadrants to another position to change the gate.

To move a gate

Select the gate and drag the gate to the desired position.

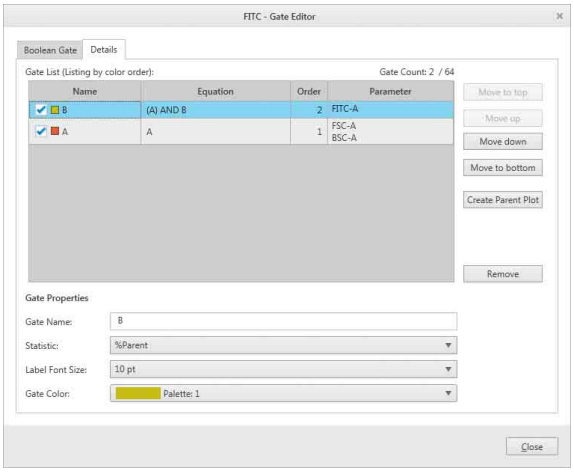
To rotate a gate

You can rotate polygon and ellipse gates.
Select a polygon or ellipse gate and drag the rotation handle to rotate the gate.



To change gate settings

Select the gate, click [Edit Gate] in the [Gate] group on the [Plot Tools] tab of the ribbon to display the [Gate Editor] dialog, and then select the [Details] tab.



For details about each item, see "[Gate Editor] dialog" (page 127).

To delete a gate

Select the gate to be deleted, then click [Remove Gate] in the [Gate] group on the [Gate Tools] tab of the ribbon.
Alternatively, right-click the gate and select [Remove] from the context menu.
You can also delete a gate using the Delete key on the keyboard.

Note

Deleting a gate will delete all data and statistics for the gate, including all child gates and plots derived from the gate.

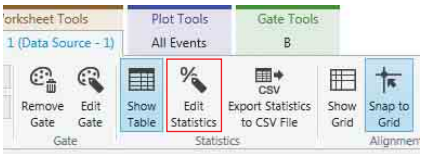
Displaying Statistics

You can change the statistics items displayed for each gate in the [Gates and Statistics] table.

For details about statistics, see "[Gates and Statistics] Table" (page 131).

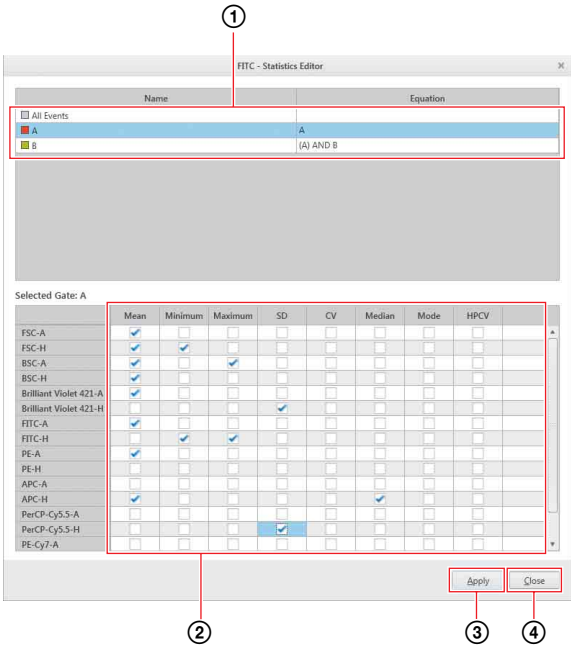
1 Click [Edit Statistics] in the [Statistics] group on the [Worksheet Tools] tab of the ribbon.

Alternatively, right-click the [Gates and Statistics] table and select [Open Statistics Editor] from the context menu.



The [Statistics Editor] dialog appears.

2 Specify the items to be displayed in the [Gates and Statistics] table.



① Select a gate.
The statistics values for the population in the selected gate are displayed in the lower area.

② Place a check mark in the checkboxes for the statistics you want to display for each parameter.

③ Click [Apply] to update the statistics displayed in the [Gate and Statistics] table.

④ Click [Close] to close the dialog.

To change the width of columns

Drag the right edge of a table column to change the width of the column.

To change the display order of columns

Select the title of the column to move, and drag it to the desired location.

Tip

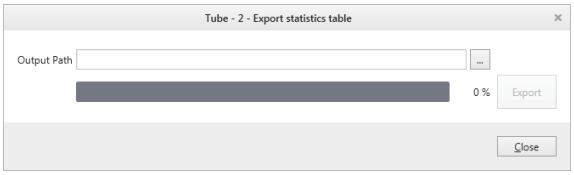
The [Name] column cannot be moved.

To export statistics as a CSV file

1 Click [Export Statistics to CSV File] in the [Statistics] group on the [Worksheet Tools] tab of the ribbon.

The [Exporting statistics table] dialog appears.

2 Click ... (Browse) to specify the folder to save the CSV file, then click [Export].



The CSV file is exported.

3 Click [Close] to close the dialog.

Sorting

Chapter 6

This chapter describes the various sorting modes and detailed sorting criteria.

The SH800 can sort selected populations of cells at high speed. It sorts droplets containing targeted events, selected using gates on plots in the worksheet, into left and right output streams (two-way sorting). Sorted droplets can be collected in 5 ml round tubes, 15 ml conical tubes, and on multi-well plates. In addition, gates can be combined using Boolean logic to uniquely identify a population for sorting using multiple attributes, providing powerful sorting capabilities.

Sorting Mode

Overview

In an ideal sort, cells travel past the optical detection point in single file and are sorted one cell at a time in separate droplets. In practice, however, cells appear randomly and can occur in close proximity within the same droplet or in adjacent droplets (called coincidences).

When a cell is detected during sorting, it is generally not possible to know with great accuracy if the signal represents the passage of a large single cell, small multiple cells adhering to one another, or other non-cellular material. Accordingly, it is more common to refer to each signal detection not as a cell, but as an event.

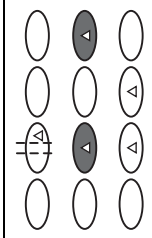
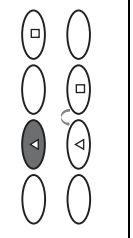
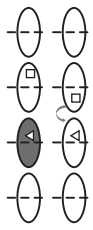
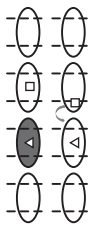
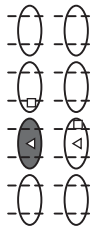
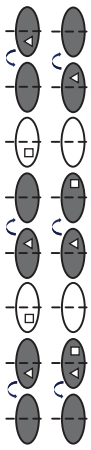
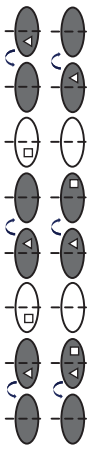
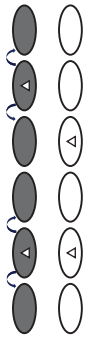
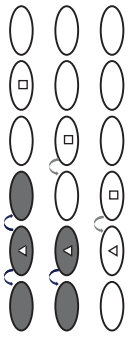
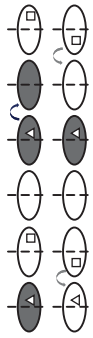
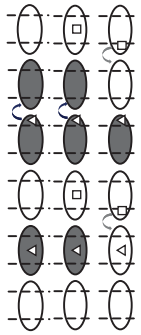
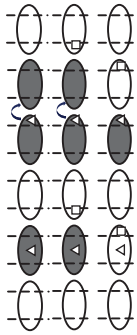
The sorting mode sets the rules used when sorting coincidences. These rules determine the sorting decision for each event based on the type of event and the distance between adjacent events. For example, a decision might be made to sort an event regardless of an adjacent unwanted event if the goal is to isolate as many cells as possible from a small population. Conversely, a decision might be made to not sort an event if there is an adjacent unwanted event that could reduce the purity of the sorted population.

Tip

The trigger threshold setting in the [Detector & Threshold Settings] dialog *(page 79)* can be critical for effective sorting. If the threshold level is set too high, small cells and cell debris (noise) that fall below the threshold value may be unintentionally sorted together with events above the trigger threshold, depending on the sorting mode setting. To increase sorting purity, it is recommended that the trigger threshold value be set to a relatively low value.

The SH800 supports the following eight sorting modes for obtaining the desired sorting results. The modes provide different levels of purity[1)] and yield[2)]. Note that the droplets that are sorted in each sorting mode vary depending on the size of the nozzle of the sorting chip.

Single Cell ← Increasing purity **Increasing yield →**

	Single Cell *(page 95)*	Ultra Purity *(page 97)*	Purity *(page 97)*	Semi-Purity *(page 96)*	Normal *(page 96)*	Semi-Yield *(page 98)*	Yield *(page 98)*	Ultra-Yield *(page 99)*
100 µm sorting chip								
Number of droplets sorted	1	1	1	1	1			
130 µm sorting chip								
Number of droplets sorted	3	1 to 3	1 or 2	1 or 2	1 or 2	1 or 2	2	3

△: Target cell, □: Non-targeted (conflicting) cell

: Sorted droplet

: Waste droplet

: Multi-drop inclusion

: Multi-drop exclusion

-----: Sorting regions

1) Purity measures the proportion of target cells collected as a percentage of all cells collected.
2) Yield measures the proportion of target cells collected as a percentage of target cells in the sample. It indicates the percentage of the target population that is collected.

A high-degree of purity of targeted cells can be obtained if the targeted cell events occur in isolation from other unwanted events. In this case, the droplets containing the targeted events are sorted, while all unwanted events and empty droplets are passed to waste disposal. In practice, however, there is a trade-off to be made between yield, recovery, and purity.

Sorting is not an exact science. There are several factors that can affect the outcome. The position of a cell within a droplet can only be determined within a certain degree of accuracy, fluctuations can occur in the fluid flow due to the passage of large cells or variations in the sheath fluid pressure, and the droplet charging pulse timing may be slightly out of phase with droplet formation.

- If a targeted event appears close to the top or bottom edge of a droplet, the probability of correct recovery is reduced. The targeted event may actually be output in an adjacent droplet. One option is to sort successive droplets in order to catch the event to increase yield and recovery.
- If a targeted event appears close to the edge of a droplet and an unwanted event occurs in the adjacent droplet, or vice versa, the decision whether to sort the droplets or not depends on the goal: isolating a pure population or recovering as many targeted cells as possible. Sorting the targeted event will increase the yield but with a subsequent reduction in purity. Conversely,

rejecting the targeted event will maintain high purity but with reduced yield. Likewise, a similar decision occurs if a targeted event and an unwanted event appear in the same droplet.

- If two, different targeted events for collection in separate collection tubes appear in the same droplet or close to one another in adjacent droplets, the sorting decision must determine which of the two targeted events is sorted correctly or whether to abort sorting of the two events.
- If two or more cells, including target and/or unwanted cells, appear at the optical detection point in very close proximity, a single event will be triggered if the detected signal does not fall below the threshold level between cells. In this case, it is impossible to distinguish the event as separate cells in a single-pass sort. If the event contains targeted and unwanted cells, it will exhibit some of the characteristics of both types of cell. If the sort criteria only define a limited subset of targeted cell characteristics, the event may satisfy that criteria and be sorted accordingly, with a corresponding reduction in purity due to the presence of the unwanted event. Sorting using multiple gates to isolate target cells for multiple characteristics can help isolate targeted events.

In addition, hardware-related factors can affect sorting. If two or more cells, including target and/or unwanted cells, appear at the optical detection point in reasonably close proximity, separate events will be triggered if the detected signal falls below the threshold level between cells. However, if the acquisition electronics are not yet ready to receive the second event, the physical presence of the second event is detected but the event is not analyzed. This time is called the dead time. These events are aborted automatically and cannot be sorted, reducing the potential yield.

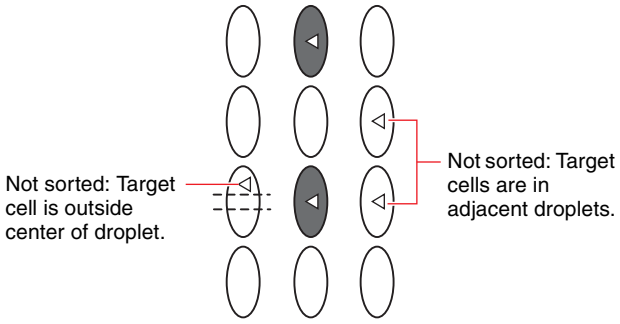
From this discussion, it can be seen that sorting only a single targeted population where the targeted events occur in the center of droplets that contain no other unwanted events will always produce the best sort results in terms of purity. Sorting all events for a single targeted population will always produce the best results in terms of yield.

Single Cell Mode

In Single Cell mode, only droplets containing a single targeted cell within the center region of the cell are sorted (adjacent droplets are also sorted when using a 130 µm sorting chip) if the adjacent droplets are empty. If an adjacent droplet contains the same type of target cell, the droplet is not sorted.

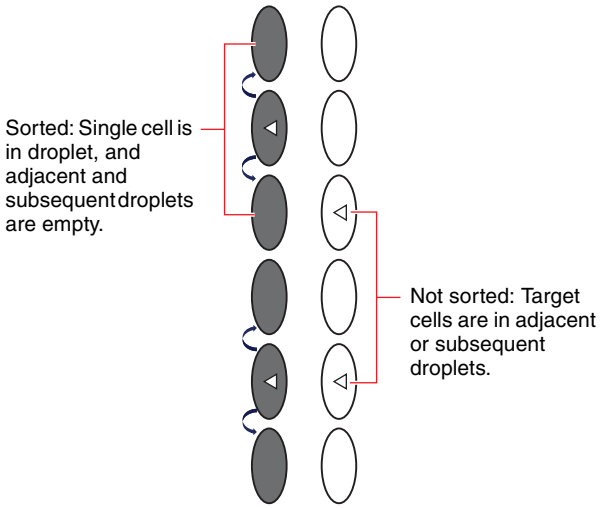
100 µm sorting chip

A droplet is sorted if it contains a single targeted event located within the center region of the droplet and both of the adjacent droplets are empty.
One droplet is sorted for each event matching the sorting criteria.



130 µm sorting chip

A droplet is sorted if it contains a single targeted event and the adjacent and subsequent droplets on both sides are all empty.
Three droplets are sorted for each event matching the sorting criteria.

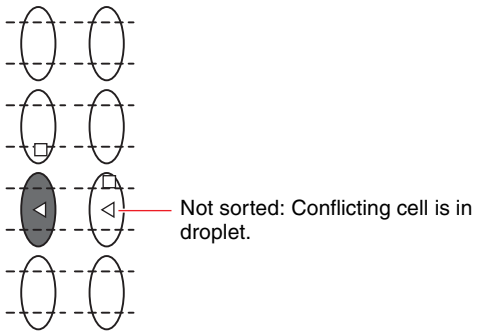

Sorted: Single cell is in droplet, and adjacent and subsequent droplets are empty.
Not sorted: Target cells are in adjacent or subsequent droplets.

Normal Mode

Normal mode is the default sorting mode.

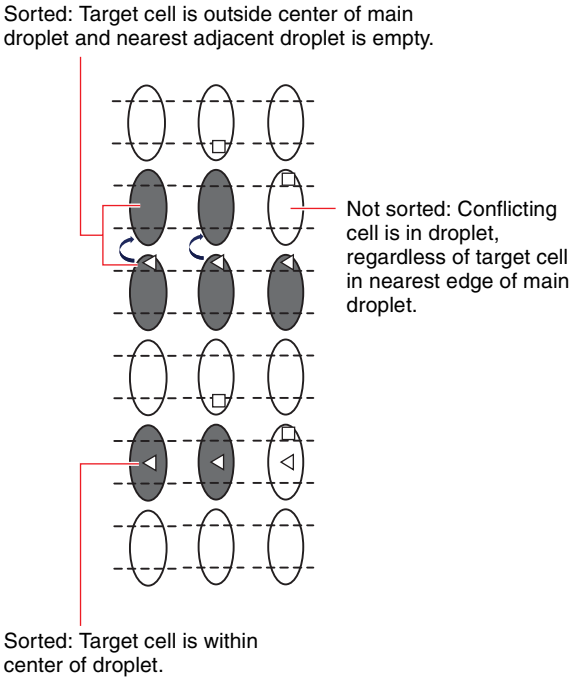
100 µm sorting chip

A droplet is sorted if it contains one or more targeted events of a single type.
One droplet is sorted for each event matching the sorting criteria.



130 µm sorting chip

A droplet is sorted if it contains one or more targeted events of a single type. In addition, if the targeted event is near the edge of the droplet, the nearest adjacent droplet is also sorted if that droplet is empty or non-conflicting.
One or two droplets are sorted for each matching sorting criteria.

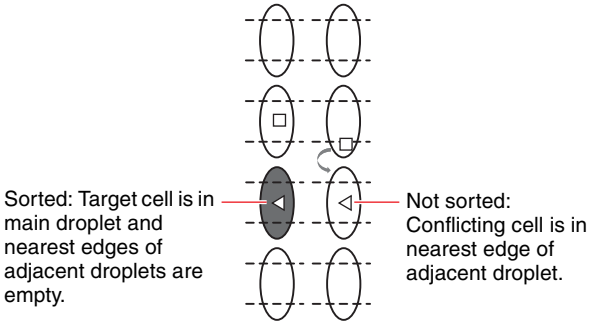

Sorted: Target cell is outside center of main droplet and nearest adjacent droplet is empty.
Not sorted: Conflicting cell is in droplet, regardless of target cell in nearest edge of main droplet.
Sorted: Target cell is within center of droplet.

Semi-Purity Mode

In Semi-Purity mode, the purity achieved is higher than in Normal mode.

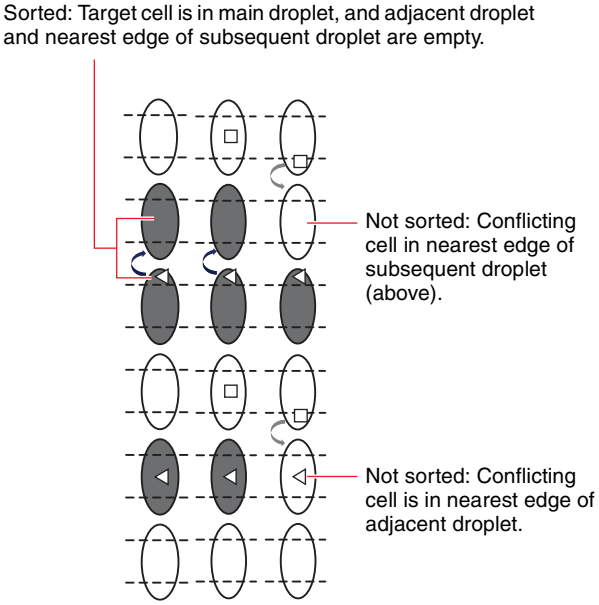
100 µm sorting mode

A droplet is sorted if it contains one or more targeted events of a single type and the nearest edge region of both of the adjacent droplets is empty or non-conflicting.
One droplet is sorted for each event matching the sorting criteria.


Sorted: Target cell is in main droplet and nearest edges of adjacent droplets are empty.
Not sorted: Conflicting cell is in nearest edge of adjacent droplet.

130 µm sorting mode

A droplet is sorted if it contains one or more targeted events of a single type and the nearest edge region of both of the adjacent droplets is empty or non-conflicting. In addition, if the targeted event is near the edge of the droplet, the nearest adjacent droplet is also sorted if the nearest edge region of the subsequent droplet is empty or non-conflicting.
One or two droplets are sorted for each event matching the sorting criteria.

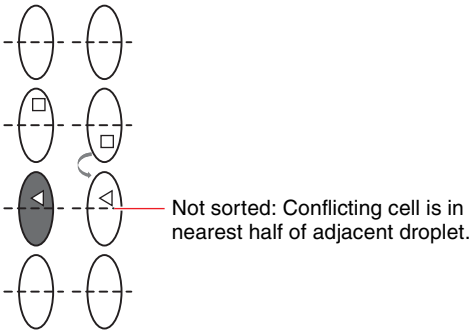

Sorted: Target cell is in main droplet, and adjacent droplet and nearest edge of subsequent droplet are empty.
Not sorted: Conflicting cell in nearest edge of subsequent droplet (above).
Not sorted: Conflicting cell is in nearest edge of adjacent droplet.

Purity Mode

In Purity mode, cells are sorted with high purity.

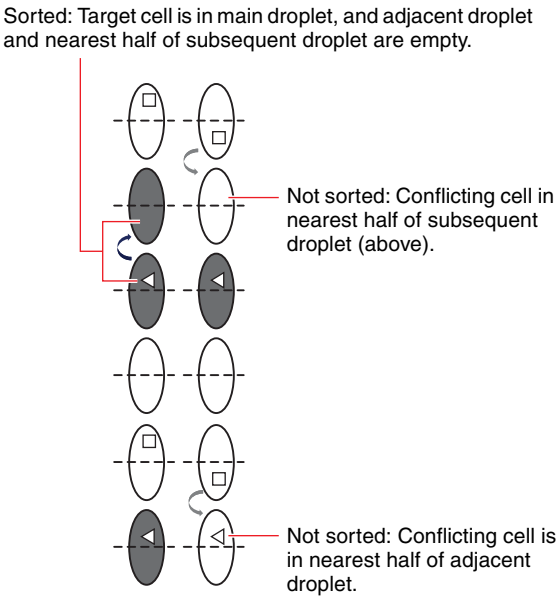
100 µm sorting chip

A droplet is sorted if it contains one or more targeted events of a single type and the nearest edge region of both of the adjacent droplets is empty or non-conflicting.
One droplet is sorted for each event matching the sorting criteria.



130 µm sorting chip

A droplet is sorted if it contains one or more targeted events of a single type and the nearest half of both of the adjacent droplets is empty or non-conflicting. In addition, if the targeted event is in one half of the droplet, the nearest adjacent droplet is also sorted if the nearest half of the subsequent droplet is empty or non-conflicting.
One or two droplets are sorted for each event matching the sorting criteria.

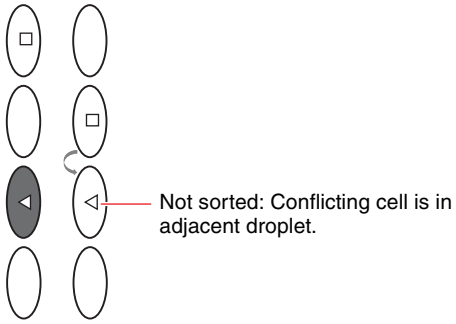

Sorted: Target cell is in main droplet, and adjacent droplet and nearest half of subsequent droplet are empty.
Not sorted: Conflicting cell in nearest half of subsequent droplet (above).
Not sorted: Conflicting cell is in nearest half of adjacent droplet.

Ultra Purity Mode

In Ultra Purity mode, cells are sorted with the highest purity.

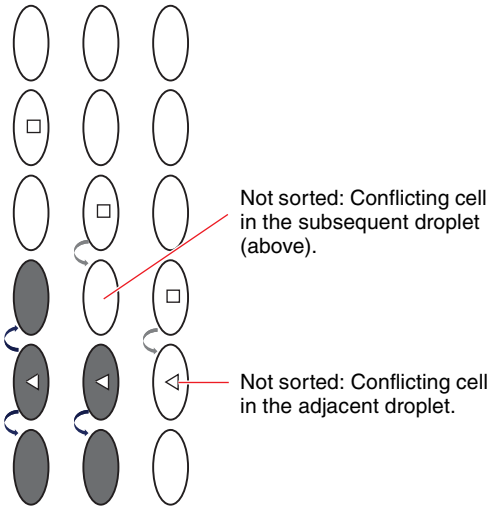
100 µm sorting chip

A droplet is sorted if it contains one or more targeted events of a single type and the both of the adjacent droplets are empty or non-conflicting.
One droplet is sorted for each event matching the sorting criteria.



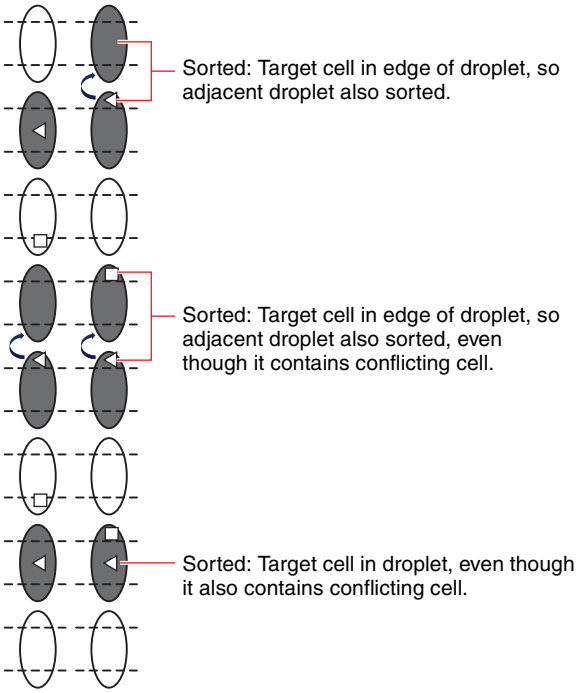
130 µm sorting chip

A droplet is sorted if it contains one or more targeted events of a single type and the both of the adjacent droplets are empty or non-conflicting. In addition, the adjacent droplet is also sorted if the subsequent droplet is empty or non-conflicting.
One, two, or three droplets are sorted for each event matching the sorting criteria.



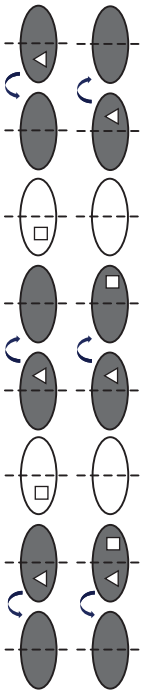
Semi-Yield Mode

In Semi-Yield mode, a droplet is sorted if it contains one or more targeted events, even if the droplet contains conflicting events. In addition, if a targeted cell is near the edge of the droplet, the adjacent droplet is also sorted, even if it too contains conflicting events.
One or two droplets are sorted for each event matching the sorting criteria.


Sorted: Target cell in edge of droplet, so adjacent droplet also sorted.
Sorted: Target cell in edge of droplet, so adjacent droplet also sorted, even though it contains conflicting cell.
Sorted: Target cell in droplet, even though it also contains conflicting cell.

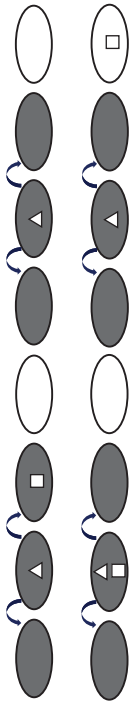
Yield Mode

In Yield mode, a droplet is sorted, together with the adjacent droplet nearest to the target event, if it contains one or more targeted events, even if the droplets contain conflicting events.
Two droplets are sorted for each event matching the sorting criteria.



Ultra Yield Mode

In Ultra Yield mode, a droplet is sorted, together both adjacent droplets, if it contains one or more targeted events, even if the droplets contain conflicting events. Three droplets are sorted for each matching sorting criteria.



Adjusting the Sort Position (SH800ZP only)

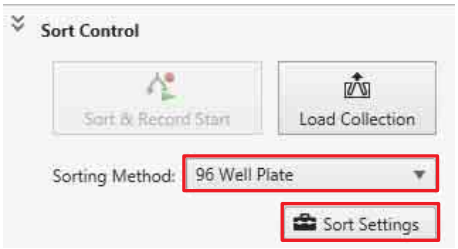
You can adjust the position where droplets fall within wells when sorting into a multi-well plate.
The description in this section shows a 96-well plate as an example. The operation is the same for all other multi-well plates.
When sorting into a 384-well plate or 384-well PCR plate, always adjust the sort position before starting.

Tip

The sort position cannot be adjusted when running 2-way tube sorting.

1 Place a cover over the multi-well plate so that you can see the position where droplets fall.

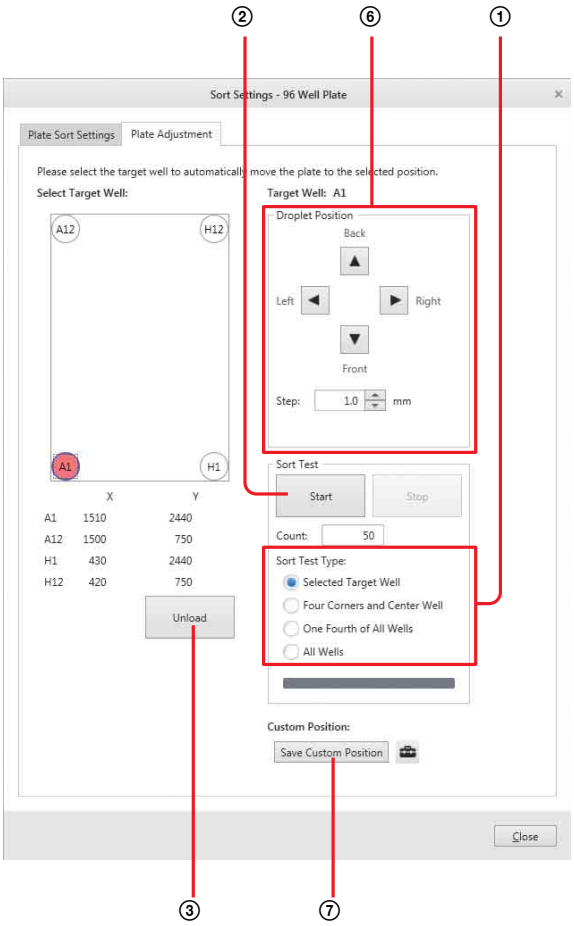
2 Select a multi-well plate sorting method in [Sorting Method] in the [Sort Control] pane, and click [Sort Settings].



The [Sort Settings] dialog appears.

3 Adjust the sort position on the [Plate Adjustment] tab.

Adjust the position where droplets fall by visually monitoring the top of the multi-well plate.



① Select the type of test sorting in [Sort Test] > [Sort Test Type].
- [Selected Target Well]: Droplets fall in the well position selected in [Select Target Well]. Select this option to test sort into a single well at a time. When selected, select the well into which droplets should fall in [Select Target Well].
- [Four Corners and Center Well]: Droplets fall into the four corner wells and the center well.
- [One fourth of All Well]: Droplets fall into 1/4 of all wells.
- [All Well]: Droplets fall into all wells.

② Click [Start] in [Sort Test].
Change the number of events to be sorted into each well in [Count], as required.
Droplets start to fall in the position selected in step ①.

③ Click [Unload].

④ Visually check the position of the droplets on the multi-well plate cover.

⑤ Wipe off droplets after confirming their position.

⑥ Adjust the sort position of droplets using the arrow buttons in [Droplet Position].
For example, if the position for well A1 was 1mm too far left and 1.5mm too far up:
(1) Click well A1.
(2) Move the position 1 mm to the right using ▶ and 1.5 mm down using ▼ in [Droplet Position].
(3) Click [Start] in [Sort Test].
(4) Click [Unload].
(5) Visually check the position of the droplets on the multi-well plate cover.
(6) Repeat the procedure for wells A12 and H12, as required.

⑦ To set the adjusted position as the default, click [Save Custom Position].

Tip

Clicking 🧰 displays the [User Preference] dialog *(page 105)* for setting user default preferences.

4 When finished, click [Close] to close the dialog.

Index Sorting

You can associate the cells sorted in each well with the data points for those cells on plots. Using the index sorting function allows you to select a specific well and view the data for cells collected in that well.
This section describes sorting using a 96-well plate, but the procedure is identical for sorting onto 8-well strips or 384-well plates.

Tip

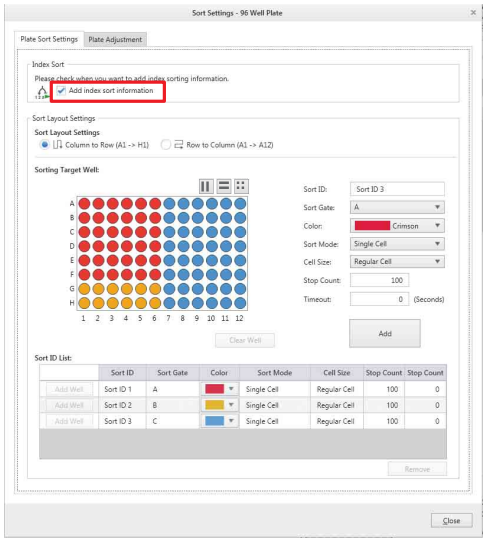
Index sorting is not available for 2-way tube sorting.

Configuring Index Sorting

1 Select a sorting method in [Sorting Method] in the [Sort Control] pane, and click [Sort Settings].

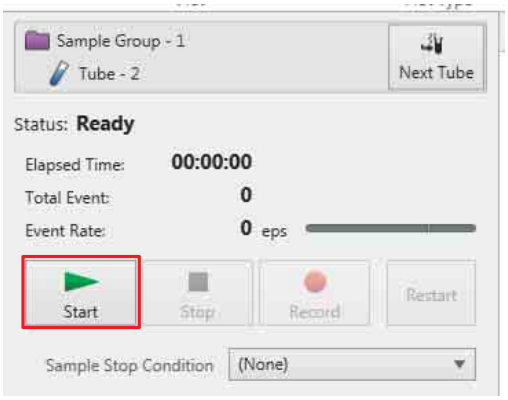
The [Sort Settings] dialog appears.

2 Place a check mark in [Add index sort information] on the [Plate Sort Settings] tab.



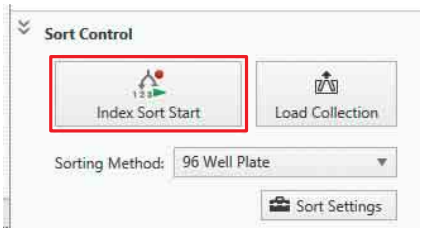
3 When finished configuring other items, click [Close] to close the dialog.

4 Click [Start] on the data acquisition control pane to start acquiring data.



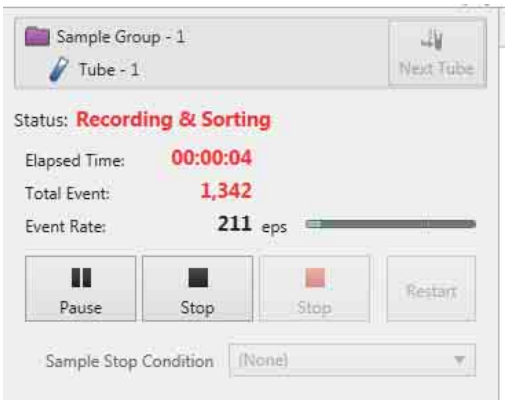
5 Click [Index Sort Start] on the [Sort Control] pane to start sorting.

When configured for indexed sorting, “Index” is displayed on the button.



Tip

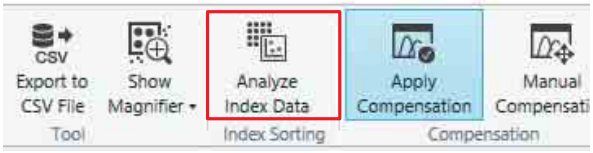
Data is acquired and cells sorted automatically in index sorting.



Analyzing Index Sort Data

This section describes how to analyze data acquired during index sorting.
This function allows you to examine the position on plots of cells sorted in a specific well.

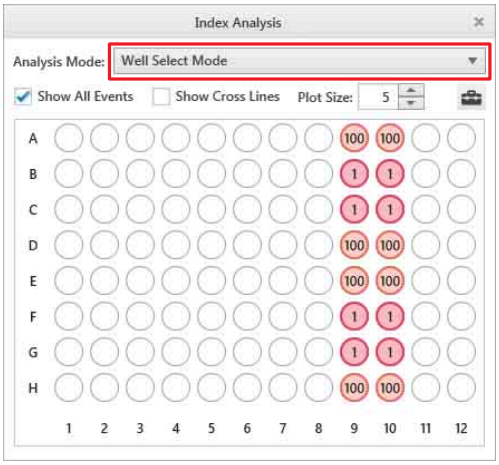
1 Click [Analyze Index Data] in the [Index Sorting] group on the [Worksheet Tools] tab of the ribbon.



The [Index Analysis] dialog appears.

2 Select a mode in [Analysis Mode].

The wells displayed vary depending on the selected mode.



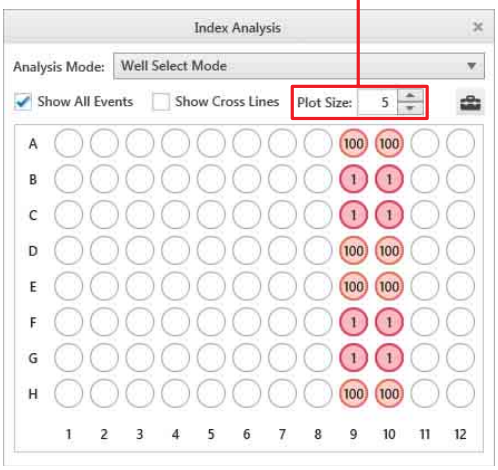
[Well Select Mode]: Select this mode when you want to display the position on plots of cells sorted in each well. You can select multiple wells at the same time.

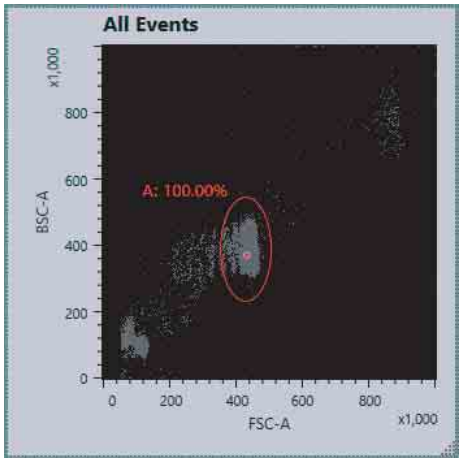
[Gate Select Mode]: Select this mode to display only those wells encompassed by the selected gate. You can also select wells to display the position of cells in those wells on plots.

If [Well Select Mode] is selected

Displays all wells in the multi-well plate. Clicking a well in the dialog displays the position on plots of the cells collected in that well.
Placing a check mark in [Show All Events] displays all data acquired during sorting (includes events that were sorted and events that were not sorted) on plots.

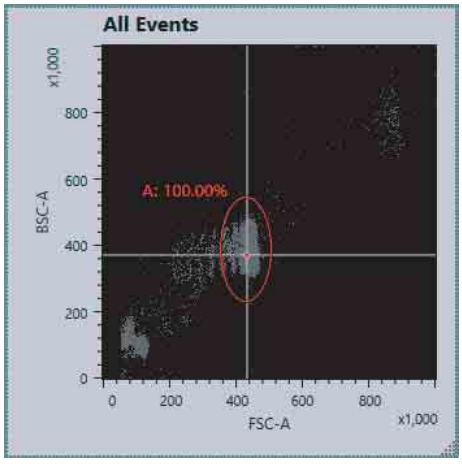
Specifies the size of the dot displayed on plots indicating the data position.





To view the dot position accurately

Placing a check mark in [Show Cross Lines] displays horizontal and vertical lines intersecting the dot position for more accurate positioning.

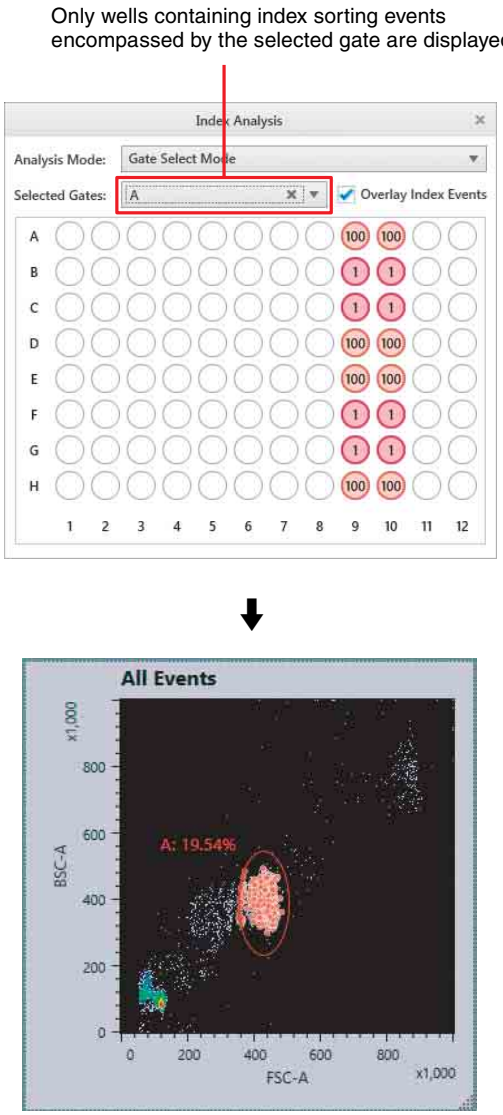


Tip

Clicking displays the [User Preference] dialog *(page 105)* for setting user default preferences.

If [Gate Select Mode] is selected

Displays only those wells that contain events encompassed by the gate selected in [Selected Gate]. Placing a check mark in [Overlay Index Events] displays the positions of only the cells in the selected gate on plots.



Tip

Clicking displays the [User Preference] dialog *(page 105)* for setting user default preferences.

Adjusting Sort Parameters Manually (Experienced Users)

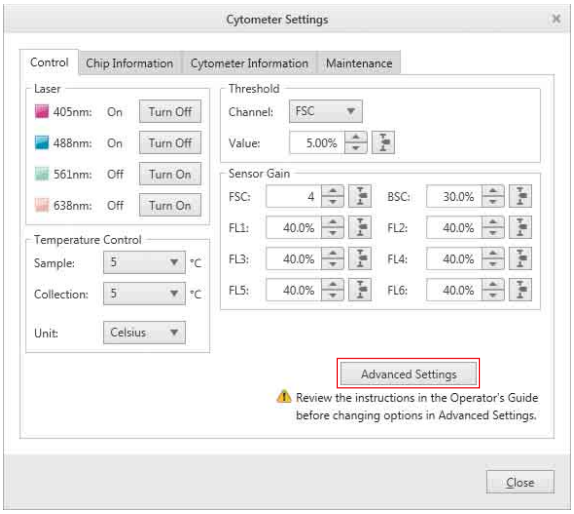
During startup, the SH800 automatically measures and calibrates the sorting parameters for sorting using automatic setup beads. However, there may be cases where you wish to override the automatic control system and adjust the sorting parameters manually.

These parameters are for experienced operators who are already familiar with manual sorting controls. The manual controls are located in the [Advanced Settings] window.

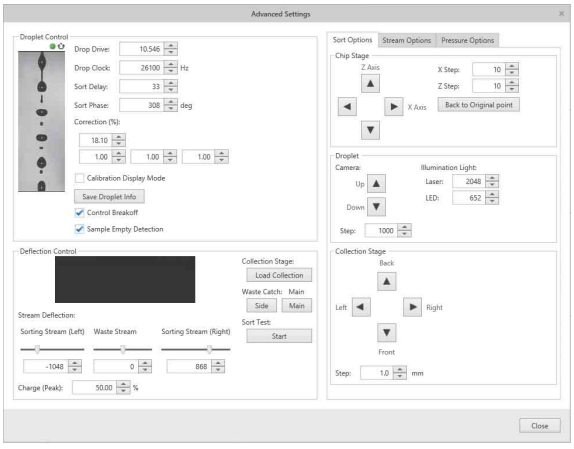
1 Click [Settings] on the [Cytometer] tab of the ribbon.

The [Cytometer Settings] dialog appears.

2 Click [Advanced Settings].



The [Advanced Settings] dialog appears.



For details about each item, see "[Advanced Settings] dialog" (page 122).

Window Description

Chapter 7

Tips

- The menus and buttons displayed vary depending on the user account privileges.
- The screenshots displayed in this document may vary from the actual software.

[File] Window

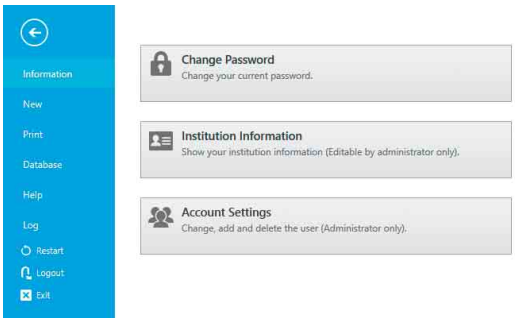
The [File] window (Backstage View) is displayed by clicking the [File] tab of the ribbon.

For details about [Restart], [Logout], and [Exit], see "[File] window" (page 26).

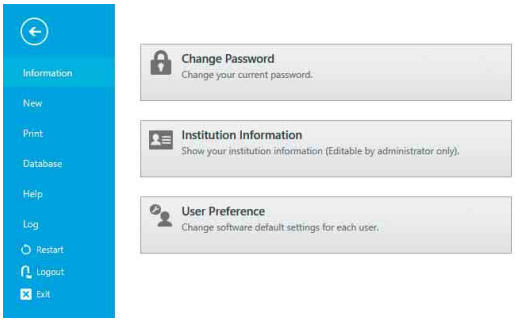
[Information] window

The [Information] window is displayed by clicking [Information] in the menu on the left side.
The [Information] window is used to change login passwords, set institution information, and add/modify user accounts.

Example: Administrator account login



Example: User account login



[Change Password]: Allows you to change the password used to log in to SH800 Software.
For details, see *"Changing Passwords" (page 39)*.

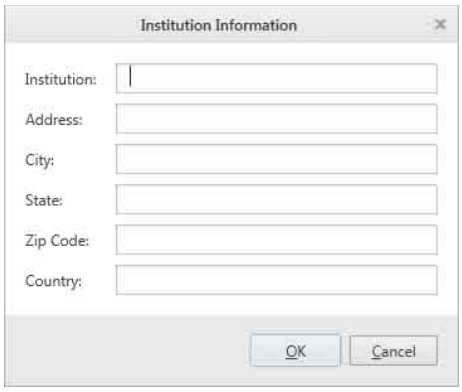
[Institution Information]: Allows you to view information on the institution using the unit.
You can also configure settings if you are logged in using an Administrator account. Settings cannot be configured if you are logged in using a general user account.
Click this button to display the [Institution Information] dialog *(page 105)*.

[Account Settings]: Displays the [Account Settings] dialog *(page 38)* for managing user accounts for SH800 Software. This button is displayed for Administrator accounts only.

[User Preference]: Displays the [User Preference] dialog *(page 105)* for specifying software preferences, which can be specified independently by each user. This button is displayed for Administrator accounts only.

[Institution Information] dialog

The [Institute Information] dialog is displayed by clicking [Information] > [Institute Information] on the [File] tab of the ribbon.
The [Institution Information] dialog is used to specify information about the institution using the unit. Only administrators can modify this information.



[Institution]: Enter the name of the institution.
[Address]: Enter the street address of the institution.
[City]: Enter the name of the city or town.
[State]: Enter the name of the state or province.
[Zip Code]: Enter the postal code number.
[Country]: Enter the name of the country or region.
[OK]: Applies the settings and closes the dialog.
[Cancel]: Cancels the settings and closes the dialog.

[User Preference] dialog

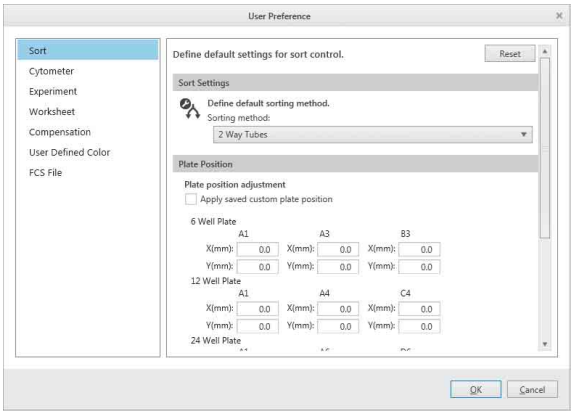
The [User Preference] dialog is used to specify software preferences, unique to each user. User preferences are loaded automatically when a user logs in, saving having to configure the instrument you want to perform acquisition, analysis, or sorting.
The [User Preference] dialog is displayed by clicking [Information] > [User Preference] on the [File] tab of the ribbon.

Tip

You can click [Reset] to restore the default values for each setting.

[Sort] tab

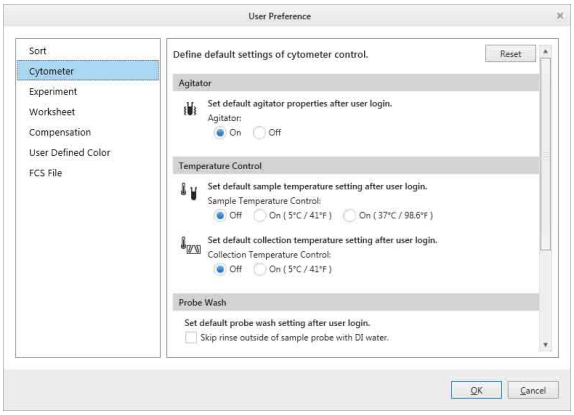
Defines default settings related to sorting.



[Sort Settings]: Specifies the default sorting configuration.
[Sort method]: Selects the sorting method. The available options vary depending on the model.
[Plate Position]: Adjusts the position of wells for each type of plate.
[Apply saved custom plate position]: Applies saved plate position adjustment values.

[Cytometer] tab

Defines default settings related to the instrument.



[Agitator]

[Agitator]: Selects the default status of the agitation unit.

[Temperature Control]

[Sample Temperature Control]: Selects the temperature of sample fluid in the sample loader.
- Off
- On (5 °C/41 °F)
- On (37 °C/98.6 °F)

[Collection Temperature Control]: Selects the temperature of sorted fluid on the collection stage.

- Off
- On (5 °C/41 °F)

[Probe Wash]

[Skip rinse outside of sample probe with DI water.]: Sets whether to skip cleaning of the outer surface of the sample probe using DI water from the DI water tank when cleaning the probe. If a check mark is placed in this checkbox, you should load a sample tube containing DI water, after cleaning the sample probe, to clean the outside of the probe.

[Area Light Control]

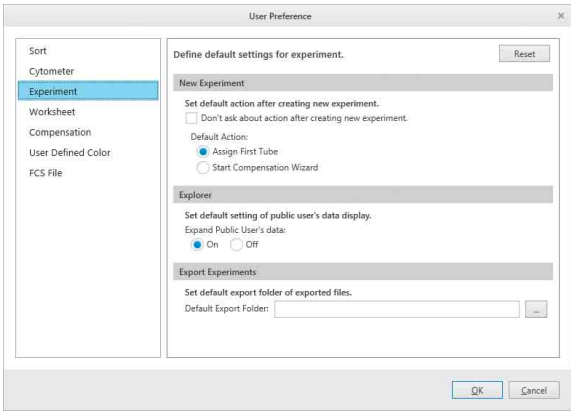
[Sample tube area light automatic control]: Specifies whether the sample loader light turns on automatically loading/unloading a sample.

- Off
- On

[Collection area light automatic control]: Specifies whether the collection area light turns on automatically when loading/unloading collection tubes.

[Experiment] tab

Defines default settings related to experiments.



[New Experiment]

[Don't ask about action after creating new experiment.]: Sets whether to display a dialog prompting you to select the next action after creating an experiment using the Blank template.

[Default Action]: Selects the default action to take after creating an experiment.

- Assign First Tube
  Assigns the first tube in preparation for data acquisition.
- Start Compensation Wizard
  Launches the [Compensation Wizard] to perform fluorescence compensation.

[Explorer]

[Expand Public User's data]: Selects whether to display the Public user data.

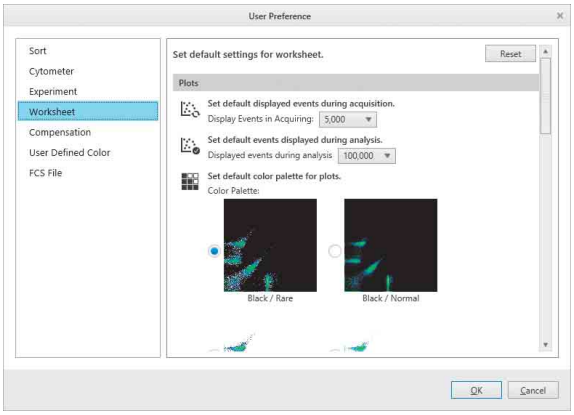
- On
- Off

[Export Experiments]

[Default Export Folder]: Specifies the destination folder used when exporting experiment data.

[Worksheet] tab

Defines default settings related to the worksheet.



[Plots]

[Display Events in Acquiring]: Selects the number of events to display during data acquisition.

[Display events during analysis]: Selects the number of events to display when analyzing data.

[Color Palette]: Selects the color palette (background color and display of outlying (rare) events).

- Black/Rare
- Black/Normal
- White/Rare
- White/Normal

[Gate]

[Label Font Size]: Selects the font size of text labels shown on gates. You can check the size in [Preview].

[Gate Line Width]: Selects the line width used to draw gates. You can check the line width in [Preview].

[Automatic zoom tracking function]: Changes the shape and size of gates on plots to track the zoom size of plots and change of axis type.

[Default Scatter Plot]

[Plot Type]: Selects the default type of plot added to a new worksheet.

- Density
- Dot Plot

[Axis Type]: Selects the default scale type of an axis.
- Linear
- Log (logarithmic)
- Biexponential

[Gate type]: Selects the default gate type.
- Ellipse
- Polygon
- Rectangle

[Index Analysis]

[Plot Size]: Specifies the size that events are displayed on plots (for emphasis) when using the index sorting function. You can check the size in [Preview].

[Alignment]

[Auto Arrange]: Selects whether to automatically align the plots and [Gates and Statistics] table to the grid of the worksheet.
- On
- Off

[Snap to Grid]: Selects whether to align the plots and [Gates and Statistics] table to the grid when moving or adding them on the worksheet.
- On
- Off

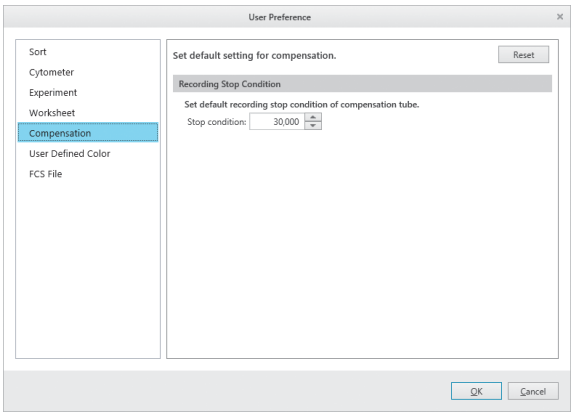
[Show Grid]: Selects whether to display the grid on the worksheet. The size of the grid is set in [Grid Size].
- On
- Off

[View]

[Expand]: Sets the zoom factor of the worksheet display.

[Compensation] tab

Defines default settings related to fluorescence compensation.

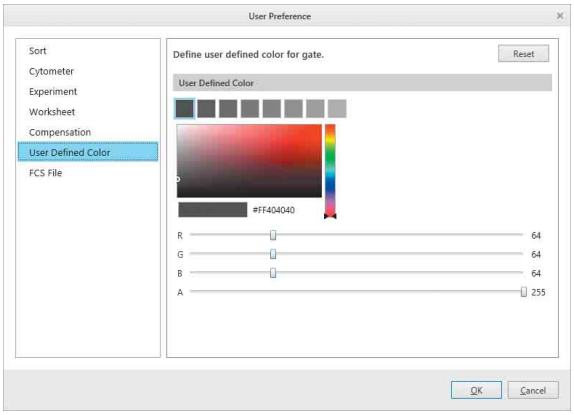


[Recording Stop Condition]

[Stop condition]: Selects the number of events before recording stops automatically.

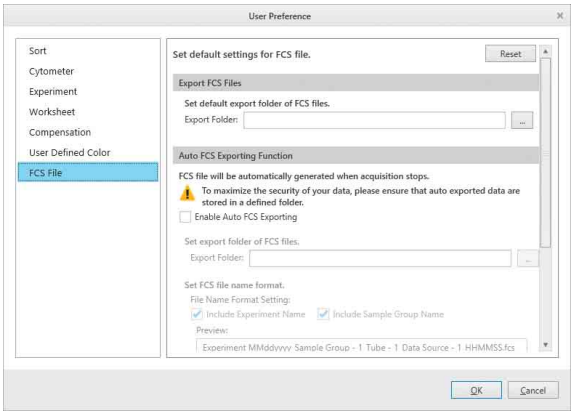
[User Defined Color] tab

Specifies custom colors for gates.



[FCS File] tab

Specifies default settings related to FCS files.



[Export FCS Files]

[Export Folder]: Specifies the destination folder used when exporting FCS files.

[Auto FCS Exporting Function]

[Enable Auto FCS Exporting]: Enables the automatic exporting of experiment data to FCS files.
Note that data security may be reduced if automatic export is enabled.

[Export Folder]: Specifies the destination folder used when exporting FCS files.

[File Name Format Setting]: Specifies the file naming format.
You can check the file name in [Preview].
- Include Experiment Name
- Include Sample Group Name

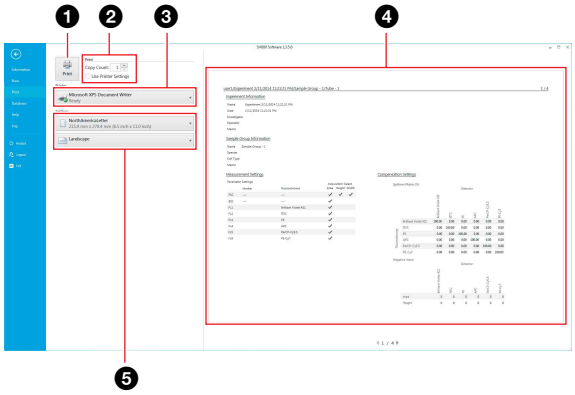
[FCS Version]: Selects the FCS file version.
- 3.0
- 3.1

[Convert BSC to SSC]: Selects whether to change the sensor name for data recorded by the "BSC" (back scatter) detector to "SSC" (side scatter) when exporting FCS files.

[Maximum Save Events]: Specifies the maximum number of events to save.

[Print] Window

The [Print] window is displayed by opening the worksheet of the tube you want to print and clicking [Print] in the menu on the left side.
The [Print] window can print analysis results, statistics, and plots displayed on the worksheet.



❶ [Print] button
Sends the print job to the printer.

❷ [Print]
Sets the number of copies to print.
Place a check mark in the [Use Printer Settings] checkbox to set advanced printer settings.

❸ [Printer]
Selects the printer for printing.

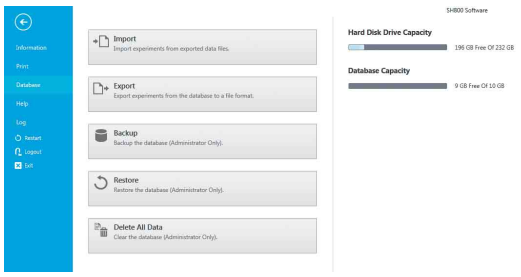
❹ Print preview area
Displays a preview image of the print job.

❺ [Settings]
Sets the paper size and page orientation.

[Database] Window

The [Database] window is displayed by clicking [Database] in the menu on the left side.
The [Database] window is used to perform experiment database operations and to check hard disk free capacity.

Example: Administrator account login



[Import]: Displays the [Import Experiment Data] dialog *(page 109)* for importing experiment data.
You can import experiment data created using another SH800 for use on this unit.

[Export]: Displays the [Export Experiment Data] dialog *(page 109)* for exporting experiment data.
You can export experiment data created on this unit for use by another SH800.

[Backup]: Displays the [Backup Database] dialog for backing up the experiment database. This button is displayed for administrator accounts only.
Backing up the database allows the data to be restored at a later time, if necessary.

For details, see "Backing Up/Restoring the Database" (page 68).

[Restore]: Displays the [Restore Database] dialog for restoring a previously backed up experiment database. This button is displayed for administrator accounts only.

For details, see "Backing Up/Restoring the Database" (page 68).

[Delete All Data]: Displays the [Delete All Data] dialog *(page 109)* for resetting the experiment database. This button is displayed for administrator accounts only.

Note

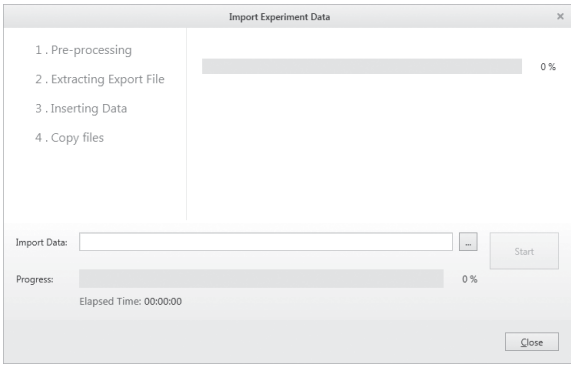
Resetting the database removes all data, including user accounts, experiments, and analysis results.

[Hard Disk Drive Capacity]: Displays the available free space on the hard disk.
A warning will appear when the free space on the hard disk falls below 50 GB. The assigning of sample tubes is disabled when the free space falls below 20 GB.

[Database Capacity]: Displays the available space on the database.
Up to 10 GB of data can be saved to the database in SH800 Software. Various types of measurement conditions and configuration settings are saved in the database. Measurement data is not stored in the database, and has no effect on the available database capacity.

[Import Experiment Data] dialog

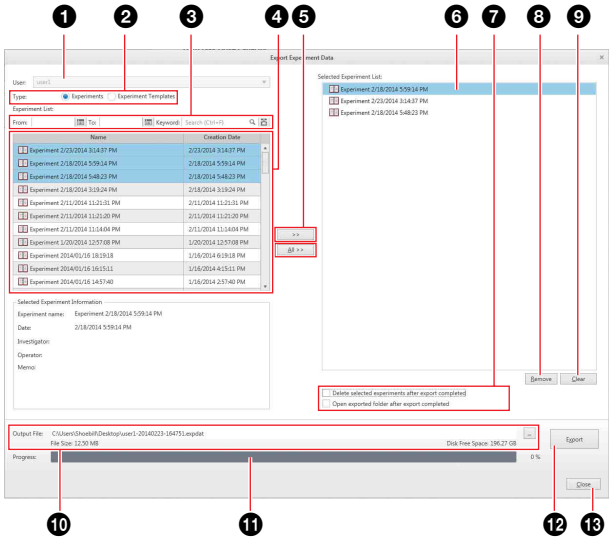
The [Import Experiment Data] dialog is displayed by clicking [Import] in the [Database] window.
The [Import Experiment Data] dialog is used to import data created on another SH800 into this instrument.



[Import Data]: Specifies the experiment data to import. Click ... (Browse) to specify a file to import.
[Progress] bar: Displays the import progress.
[Start]: Starts importing the specified data.
[Close]: Closes the dialog.

[Export Experiment Data] dialog

The [Export Experiment Data] dialog is displayed by clicking [Export] in the [Database] window.
The [Export Experiment Data] dialog is used to export data created on this unit.



❶ User
Selects the owner of the experiments to export.

❷ [Type]
Selects whether to export experiments or experiment templates.

❸ Search fields
Searches for experiments by date and keyword.

❹ Experiment list
Displays a list of experiments that satisfy the specified search criteria.

Select the experiment(s) you want to export.
Selecting an experiment in the upper list displays information about the experiment in the lower list.

❺ [>>] / [All >>]
Clicking [>>] adds the experiment data selected in the experiment data list to [Selected Experiment List].
Clicking [All >>] adds all the experiments in [Experiment List] to [Selected Experiment List].

❻ [Selected Experiment List]
Lists the experiments to be exported.

❼ [Delete selected experiments after export completed]
Deletes the exported experiments from the database after exporting has finished.
[Open exported folder after export completed]
Opens the export destination folder after exporting has finished.

❽ [Remove]
Removes the experiment selected in [Selected Experiment List] from the list.

❾ [Clear]
Clears the [Selected Experiment List].

❿ [Output File]
Specifies the export destination file. Click ... (Browse) to specify the file.

⓫ Progress
Displays the export progress.

⓬ [Export]
Starts exporting the experiment data.

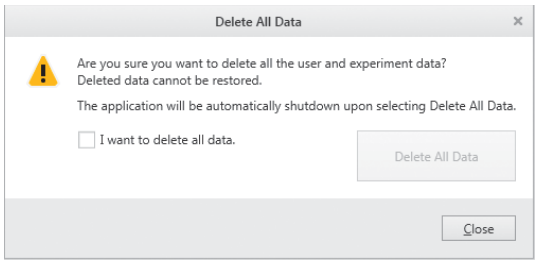
⓭ [Close]
Closes the dialog.

[Delete All Data] dialog

The [Delete All Data] dialog is displayed by clicking [Delete All Data] in the [Database] window.
The [Delete All Data] dialog is used to remove all data, including user accounts and experiments.

Notes

- Resetting the database resets all data, including user accounts, experiments, and analysis results.
- After resetting the database, SH800 Software automatically shuts down.


Delete All Data
Are you sure you want to delete all the user and experiment data?
Deleted data cannot be restored.
The application will be automatically shutdown upon selecting Delete All Data.
I want to delete all data.
Delete All Data
Close

[I want to delete all data]: Place a check mark in the checkbox to confirm you want to reset the database.
[Delete All Data]: Resets the database, deleting all data.
[Close]: Closes the dialog.

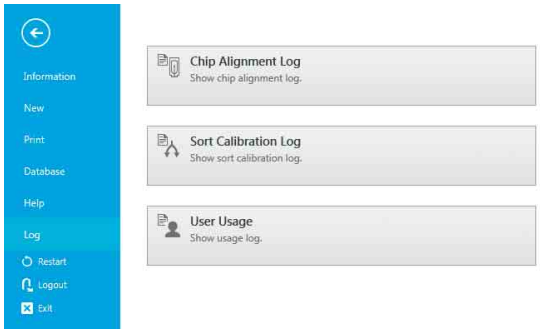
[Help] Window

The [Help] window is displayed by clicking [Help] in the menu on the left side.
The [Help] window is used to display the Operator's Guide (this document) and to check software version information.

[Open Manual]: Displays the Operator's Guide.
[SH800 Software]: Displays the SH800 Software version information.
Clicking [End User License Agreement] displays the license agreement.
[Cytometer Version]: Displays version information for instrument components.

[Log] Window

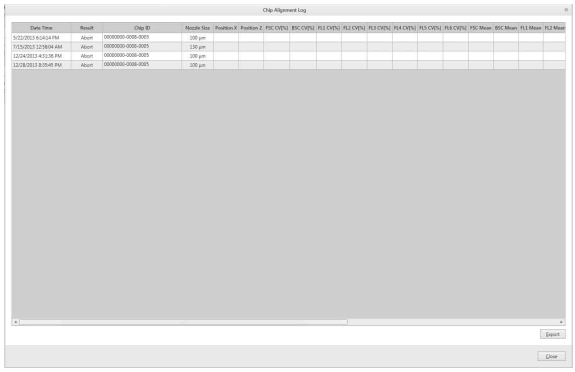
The [Log] window is displayed by clicking [Log] in the menu on the left side.
The [Log] window is used to display the chip alignment log, sort calibration log, and user usage log.



[Chip Alignment Log]: Displays the position alignment log for the sorting chip.
[Sort Calibration Log]: Displays the sort calibration log.
[User Usage]: Displays the user usage log.

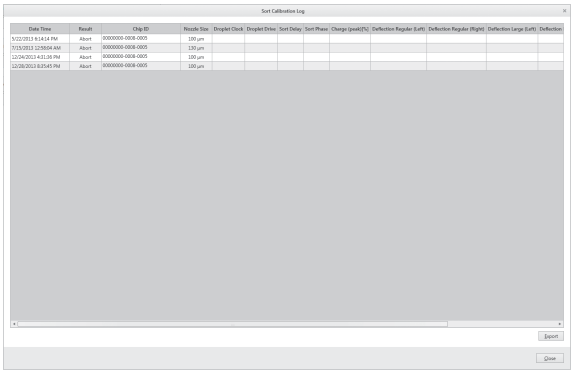
Chip alignment log

Displays the time and date when the sorting chip was aligned, together with other sorting chip information.
Clicking [Export] exports the contents of the log to a CSV-format file.



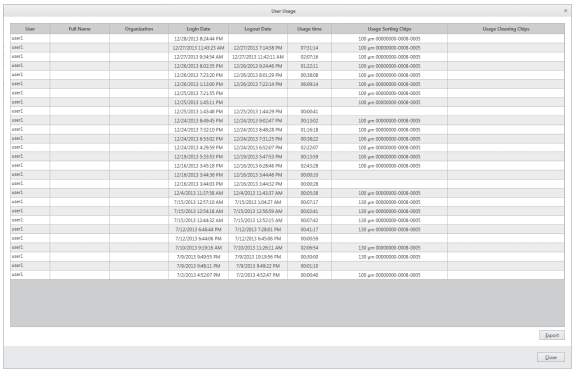
Sorting calibration Log

Displays the time and date when sorting was calibrated, together with other calibration information.
Clicking [Export] exports the contents of the log to a CSV-format file.



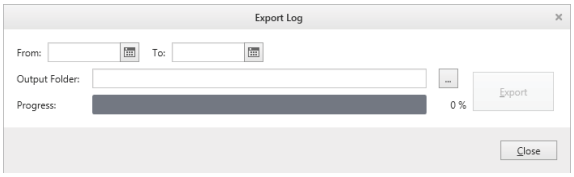
User usage log

Displays the date and time users logged in to SH800 and other user information.
Clicking [Export] exports the contents of the log to a CSV-format file.



[Export Log] dialog

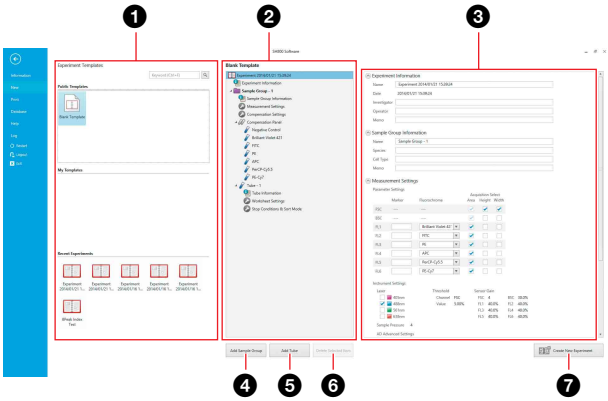
The [Export Log] dialog is displayed by clicking [Export] in the chip alignment log, sort calibration log, or user usage log dialogs.
The [Export Log] dialog is used to export the contents of the displayed log as a CSV-format file.



[From] / [To]: Specifies the interval of the log to be exported. Click to enter a date.
[Output Folder]: Specifies the output destination folder. Click to browse for a destination folder.
Progress: Displays the export progress.
[Export]: Starts exporting the log.
[Close]: Closes the dialog.

[Create Experiment] Window

The [Create Experiment] window is displayed by clicking [New] on the [File] tab of the ribbon or [New] in the [Experiment] group on the [Experiment] tab of the ribbon.
The [Create Experiment] window is used to create a new experiment using templates.



❶ [Experiment Templates] list
Displays the available experiment templates and experiments that can be used as templates.
You can search the template list by keywords.
[Public Templates]: Displays experiment templates that were created as shared templates.
[Blank Template]: An empty template.
[My Templates]: Displays private experiment templates that you have created.
[Recent Experiments]: Displays recent experiments that can be used as templates.

❷ Experiment structure
Displays the internal structure of the experiment template selected in the list.
You can add and delete sample groups and sample tubes, as required.

❸ Experiment settings
Displays configurable information and instrument settings for the experiment.
You can also edit settings after an experiment has been created.
[Experiment Information]: Specifies the name and other basic information of the experiment. The date cannot be modified.

For details about each item, see "[Experiment Information] dialog" (page 134).

[Sample Group Information]: Specifies information relating to the sample groups.

For details about each item, see "[Sample Group Information] dialog" (page 134).

[Measurement Settings]: Specifies parameter settings and instrument settings.

For details about each item, see the [Measurement Settings] dialog (page 73).

[Tube Information]: Specifies information relating to the sample tubes.

For details about each item, see "[Tube Information] dialog" (page 135).

[Compensation Settings]: Displays compensation settings.

For details about each item, see "[Compensation Settings] dialog" (page 124).

[Sort & Stop Settings]: Displays the sorting and automatic stop conditions.

❹ [Add Sample Group]
Displays the [Add Sample Group] dialog *(page 112)* for adding a sample group to the experiment structure.

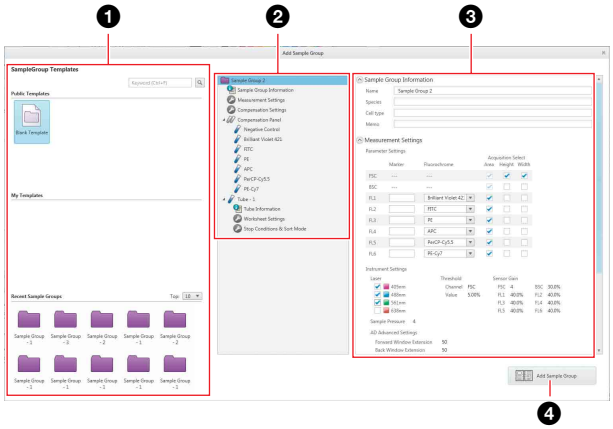
❺ [Add Tube]
Displays the [Add Tube] dialog *(page 113)* for adding a sample tube to the selected sample group in experiment structure.

❻ [Delete Selected Item]
Deletes the selected item from the experiment structure.

❼ [Create New Experiment]
Creates a new experiment with the specified settings. The experiment is added to the Experiment Explorer in the main window. The created experiment is automatically made the active experiment.

[Add Sample Group] dialog

The [Add Sample Group] dialog is displayed by clicking [Add Sample Group] in the [Create Experiment] window.
The [Add Sample Group] dialog is used to add a sample group to the experiment structure.



❶ [Sample Group Templates] list
Displays the available sample group templates and recent sample groups that can be used as templates.
You can search the template list by keywords.
[Public Templates]: Displays sample group templates that were created as shared templates.
[Blank Template]: An empty template.
[My Templates]: Displays private sample group templates that you have created.
[Recent Sample Groups]: Displays recent sample groups that can be used as templates.

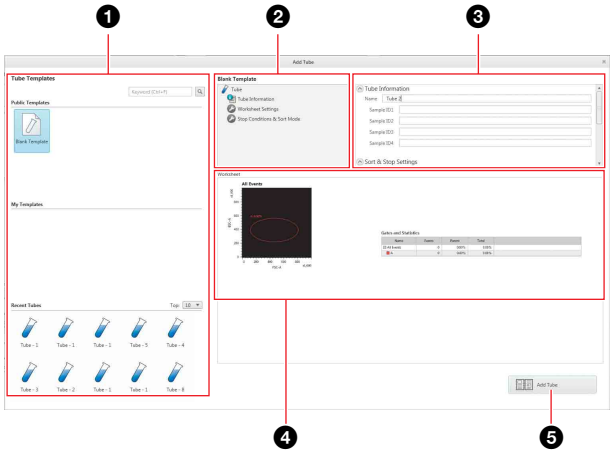
❷ Sample group structure
Displays the internal structure of the sample group template selected in the list.

❸ Sample group settings
Displays configurable information and instrument settings for the sample group.
You can also edit settings after an experiment has been created.
For details about configurations items, see *"Experiment settings" (page 111)* in the [Create Experiment] Window.

❹ [Add Sample Group]
Adds a new sample group with the specified settings to the experiment structure.

[Add Tube] dialog

The [Add Tube] dialog is displayed by clicking [Add Tube] in the [Create Experiment] window.
The [Add Tube] dialog is used to add a sample tube to the selected sample group in the experiment structure.



❶ [Tube Templates] list
Displays the available tube templates and sample tubes that can be used as templates.
You can search the template list by keywords.
[Public Templates]: Displays sample tube templates that were created as shared templates.
[Blank Template]: An empty template.
[My Templates]: Displays private tube templates that you have created.
[Recent Tubes]: Displays recent sample tubes that can be used as templates.

❷ Sample tube structure
Displays the internal structure of the sample tube template selected in the list.

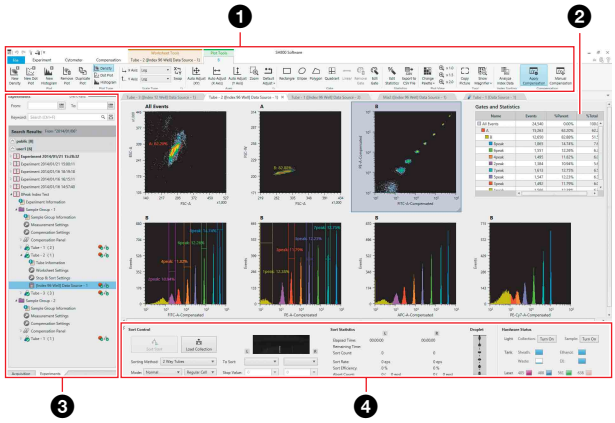
❸ Sample tube settings
Displays configurable information and instrument settings for the sample tube.
You can also edit settings after an experiment has been created.
For details about configurations items, see *"Experiment settings" (page 111)* in the [Create Experiment] Window.

❹ Worksheet
Displays the plots and gate statistics configured for the selected template or sample tube.

❺ [Add Tube]
Adds a new sample tube with the specified settings to the selected sample group in the experiment structure.

Main Window

The main window is where you acquire and record data, perform analysis, run cell sorting, and other functions.
The main window has the following configuration.



❶ Ribbon
- [File] tab *(page 104)*
- [Experiment] tab *(page 113)*
- [Cytometer] tab *(page 117)*
- [Compensation] tab *(page 124)*
- [Worksheet Tools] tab *(page 125)*
- [Plot Tools] tab *(page 129)*
- [Gate Tools] tab *(page 130)*

❷ Worksheet, [Gates and Statistics] table *(page 131)*, context menu *(page 139)*

❸ [Acquisition] tab *(page 132)* / [Experiments] tab *(page 133)*, context menu *(page 139)*

❹ Sorting control pane *(page 136)*

[Experiment] Tab (ribbon)

The [Experiment] tab of the ribbon has the following buttons.

[Target] group

Contains tool buttons for specifying the active sample tube used for data acquisition, recording, and sorting.

[Assign Tube]: Assigns the tube selected in the Experiment Explorer as the active tube for data acquisition, recording, and cell sorting.
[Next Tube]: Assigns the next tube. If there are no further tubes, it duplicates the current tube and assigns the new tube.

[Experiment] group

Contains tool buttons for creating, deleting, and performing other actions on experiments.

[New]: Displays the [Create Experiment] window *(page 111)* for creating a new experiment.
[Delete]: Deletes the experiment selected in the Experiment Explorer.
[Duplicate]: Duplicates the experiment selected in the Experiment Explorer.
[Save as Template]: Displays the [Save as Template] dialog *(page 115)* for saving the experiment selected in the Experiment Explorer as a template used when creating a new experiment.
[Send to Public]: Creates a duplicate of the selected experiment and places the duplicate under the [Public] node for sharing with other users.

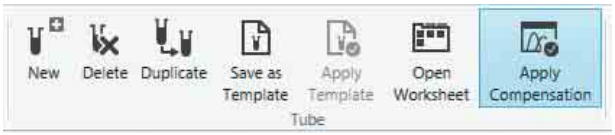
[Sample Group] group

Contains tool buttons for creating, deleting, and performing other actions on sample groups.

[New]: Creates a new, blank sample group in the experiment selected in the Experiment Explorer.
[Delete]: Deletes the sample group selected in the Experiment Explorer.
[Duplicate]: Duplicates the sample group selected in the Experiment Explorer.
[Save as Template]: Displays the [Save as Template] dialog *(page 115)* for saving the sample group selected in the Experiment Explorer as a template used when creating a new experiment.
[Apply Template]: Displays the [Apply Template] dialog *(page 115)* for selecting a template for a new sample group selected in the Experiment Explorer.

[Tube] group

Contains tool buttons for creating, deleting, and performing other actions on sample tubes.



[New]: Creates a new, blank sample tube in the sample group selected in the Experiment Explorer.
[Delete]: Deletes the sample tube selected in the Experiment Explorer.
[Duplicate]: Duplicates the sample tube selected in the Experiment Explorer.
[Save as Template]: Displays the [Save as Template] dialog *(page 115)* for saving the sample tube selected in the Experiment Explorer as a template used when adding a sample tube.
[Apply Template]: Displays the [Apply Template] dialog *(page 115)* for selecting a template for a new sample tube selected in the Experiment Explorer.
[Open Worksheet]: Opens the worksheet tab for the tube selected in the Experiment Explorer.
[Apply Compensation]: Applies fluorescence compensation to the plots on the worksheet for the tube selected in the Experiment Explorer.

[Settings and Information] group

Contains tool buttons for copying, pasting, and displaying settings and information.

[Copy] / [Paste]: Copies/Pastes the item selected in the Experiment Explorer.
[Apply to All Tubes]: Applies the worksheet settings for the tube selected in the Experiment Explorer to all sample tubes within the sample group.
Click [Worksheet Settings] for a tube in the Experiment Explorer and then click [Apply To All Tubes] to apply the same worksheet settings to the other tubes in the sample group for easy comparison during analysis.
[Show Information]: Displays the information dialog for the item ([Experiment Information], [Sample Group Information], etc.) selected in the Experiment Explorer.
You can also display the settings dialog by double-clicking [Experiment Information] and other items in the Experiment Explorer.

[Show Settings]: Displays the configuration dialog for the settings item ([Measurement Settings], [Compensation Settings], etc.) selected in the Experiment Explorer.
You can also display the settings dialog by double-clicking [Experiment Information] and other items in the Experiment Explorer.

[Show Results]: Displays the [Tube Results] dialog *(page 115)* for displaying the recording results and sorting results for the tube selected in the Experiment Explorer.
If [8 Well Strips], [Slides], [96 Well Plate], or [384 Well Plate] sorting was performed, the data source results that were recorded last are also displayed. If you click this button while a data source is selected, the [Data Source Results] dialog *(page 117)* appears, allowing you to view the results of each data source.

[Export] group

Contains a tool button for exporting recorded data for a sample tube.

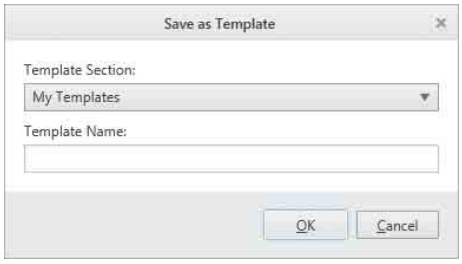
[Export to FCS Files]: Displays the [FCS File Export] dialog *(page 117)* for exporting the data recorded for the tubes in the experiment selected in the Experiment Explorer in FCS 3.0 or FCS 3.1 format.

Tip

The importing of FCS files is not supported in SH800 Software.

[Save as Template] dialog

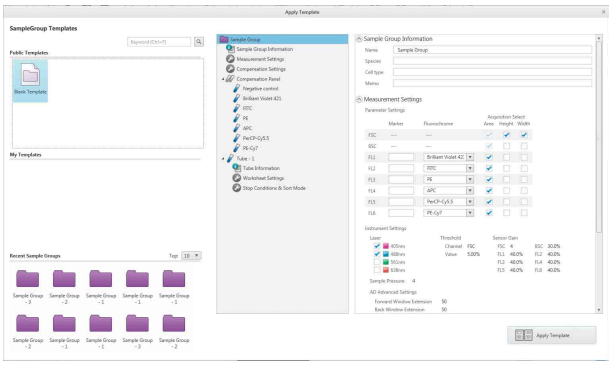
The [Save as Template] dialog is displayed by selecting an experiment, sample group, or sample tube in the Experiment Explorer and clicking [Save as Template] on the [Experiment] tab of the ribbon or by right-clicking and selecting [Save as Template] from the context menu.
The [Save as Template] dialog is used to save the selected experiment, sample group, or sample tube as a template.



For details, see "Saving an Experiment as a Template" (page 78).

[Apply Template] dialog

The [Apply Template] dialog is displayed by selecting a new sample group or sample tube in the Experiment Explorer and clicking [Apply Template] on the [Experiment] tab of the ribbon.
The [Apply Template] dialog is used to select a template to apply to the sample group or sample tube.

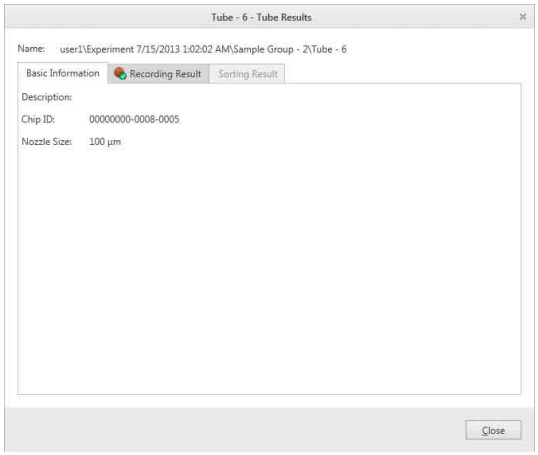


The configurable items are the same as the [Add Sample Group] dialog (page 112) or [Add Tube] dialog (page 113) for sample groups.

[Tube Results] dialog

The [Tube Results] dialog is displayed by selecting a sample tube in the Experiment Explorer and clicking [Show Results] in the [Settings and Information] group on the [Experiment] tab of the ribbon.
The [Tube Results] dialog is used to check the recording results and sorting results of the selected sample tube.

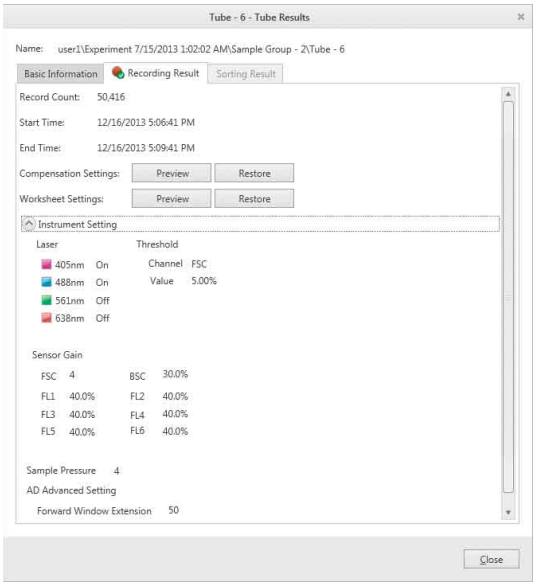
- **[Basic Information] tab**
  Displays basic information identifying the sample tube.



[Chip ID]: Displays the ID number of the sorting chip.

[Nozzle Size]: Displays the nozzle size of the sorting chip.

- **[Recording Result] tab**
  Displays recording results.



[Record Count]: Displays the number of events recorded.

[Start Time]: Displays the time when recording started.

[End Time]: Displays the time when recording ended.

[Compensation Settings]: Previews/restores fluorescence compensation settings, saved when data recording ends.

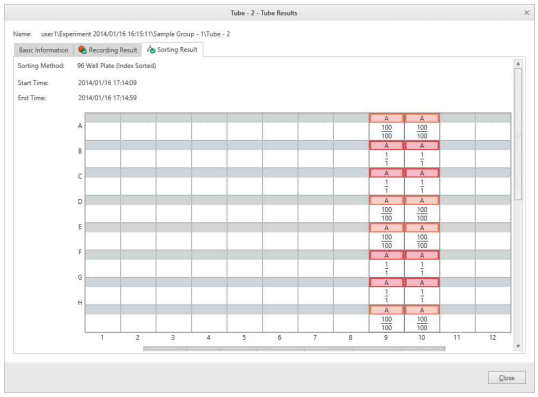
- [Preview]: Displays the fluorescence compensation settings.
- [Restore]: Restores the fluorescence compensation settings. Click [Yes] in the confirmation dialog to proceed.

[Worksheet Settings]: Previews/restores worksheet settings, saved when data recording ends.

- [Preview]: Displays the worksheet settings.
- [Restore]: Restores the worksheet settings. Click [Yes] in the confirmation dialog to proceed.

[Instrument Settings]: Displays the instrument settings.

- **[Sorting Result] tab**
  Displays the sorting results.



[Copy to Clipboard]: Copies the sorting results data to the clipboard as an image for pasting in other software, for example to produce reports.

[Save as CSV]: Saves the sorting results as CSV-format file.

[Compensation Settings]: Previews/restores fluorescence compensation settings, saved when sorting ends.

- [Preview]: Displays the fluorescence compensation settings.
- [Restore]: Restores the fluorescence compensation settings. Click [Yes] in the confirmation dialog to proceed.

[Worksheet Settings]: Previews/restores worksheet settings, saved when sorting ends.

- [Preview]: Displays the worksheet settings.
- [Restore]: Restores the worksheet settings. Click [Yes] in the confirmation dialog to proceed.

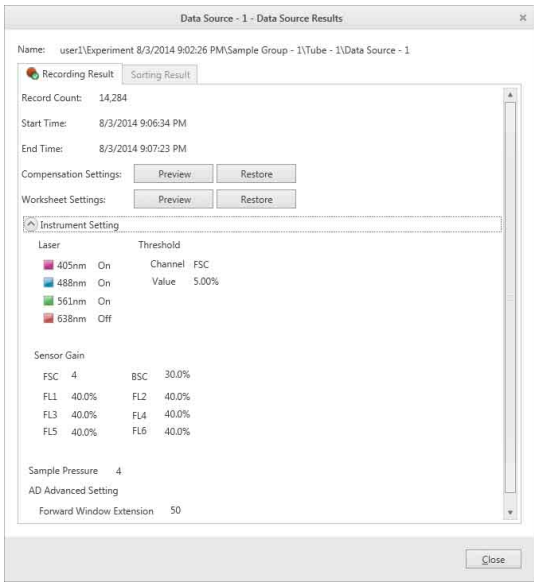
[Sort Settings]: Displays the sort settings.

[Instrument Setting]: Displays the instrument settings.

[Auto Parameters]: Displays automatically configured sorting parameters.

[Data Source Results] dialog

The [Data Source Results] dialog is displayed by selecting a data source in the Experiment Explorer and clicking [Show Results] in the [Settings and Information] group in the [Experiment] tab of the ribbon. The [Data Source Results] dialog allows you to view the results of each data source.

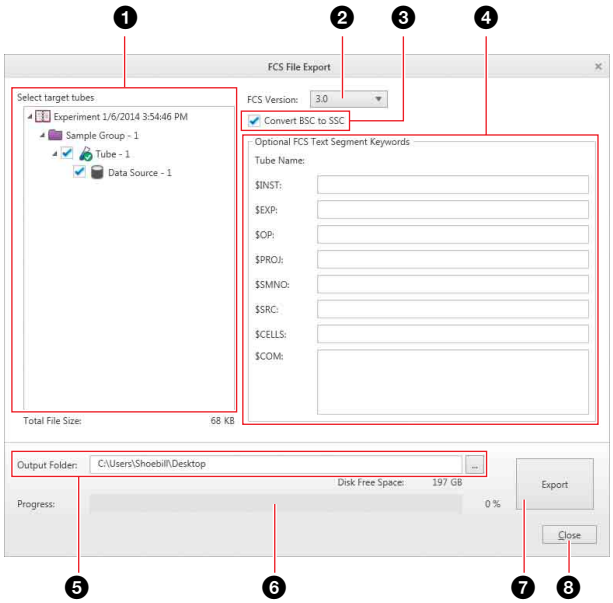


The [Data Source Results] dialog displays all the information from the [Tube Results] dialog, except the [Basic Information] tab.

For details, see "[Tube Results] dialog" (page 115).

[FCS File Export] dialog

The [FCS File Export] dialog is displayed by selecting an experiment in the Experiment Explorer and clicking [Export to FCS file] in the [Export] group on the [Experiment] tab of the ribbon or by right-clicking and selecting [Export FCS Files] from the context menu.



❶ [Select target tubes]
Selects the sample tubes to export. Place a check mark in the checkbox for each target tube.

❷ [FCS Version]
Selects the FCS file version (3.0 or 3.1).

❸ [Convert BSC to SSC]
Selects whether to change the sensor name for data recorded by the "BSC" (back scatter) detector to "SSC" (side scatter) in the exported FCS files.

❹ [Optional FCS Text Segment Keywords]
You can specify optional FCS text segment keywords, as required. Required FCS text segment keywords are exported automatically.
The following optional FCS text segment keywords can be specified.

- $INST: Institution at which data was acquired
- $EXP: Name of investigator initiating the experiment
- $OP: Name of instrument operator
- $PROJ: Name of the experiment project
- $SMNO: Specimen (e.g., tube) label
- $SRC: Source of the specimen (patient name, cell type)
- $CELLS: Description of objects measured
- $COM: Comment

❺ [Output Folder]
Specifies the export destination folder. Click ... (Browse) to specify the destination folder.
The files are named in "<tube_name>.fcs" format.

❻ Progress
Displays the export progress.

❼ [Export]
Starts the export.

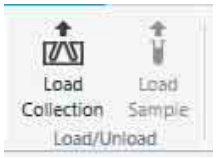
❽ [Close]
Closes the dialog.

[Cytometer] Tab (ribbon)

The [Cytometer] tab of the ribbon has the following buttons.

[Load/Unload] group

Contains tool buttons for loading and unloading sample tubes and the collection stage.



[Load Collection/Unload Collection]: Loads/unloads the collection stage in the sorting area.
[Load Sample/Unload Sample]: Loads/unloads the sample tube in the sample loader.

[Light] group

Contains tool buttons for turning the collection stage and sample loader lights on/off.

[Collection]: Turns the collection stage light on/off.
[Sample]: Turns the sample loader light on/off.

[Cleaning] group

Contains tool buttons for cleaning the fluidics system.

[Probe Wash]: Washes the sorting chip, sample line, and the inner and outer surfaces of the sample probe using sheath fluid.
[Bleach Cleaning]: Displays the [Bleach Cleaning] dialog *(page 120)* for cleaning the sorting chip, sample line, and sample probe with a Sodium Hypochlorite solution.

For details about cleaning using Sodium Hypochlorite solution, see "Cleaning the Sample Fluidics System using Bleach" (page 152).

[DI Rinse]: Displays the [DI Rinse] dialog *(page 120)* for flushing the sorting chip, sample line, and sample probe with DI water.
[Shutdown Rinse]: Displays the [Shutdown DI Rinse] dialog *(page 120)* for flushing the sorting chip, sample line, and sample probe with DI water before shutting down the instrument.

Note

After cleaning, the fluidics lines are filled with DI water to prevent the formation of salt deposits. Running data acquisition in this state may damage cells.

[De-bubble] group

Contains tool buttons for removing air bubbles from the sorting chip and sheath filter.

[Chip]: Removes air bubbles from the sorting chip.

[Sheath Filter]: Displays the [Sheath Filter De-bubble] dialog *(page 120)* for removing air bubbles from the sheath filter.

Tip

The sheath filter de-bubble function should be run at least three times after changing the sheath filter.

[Auto Calibration] group

Contains tool buttons for aligning the chip and calibrating sorting.

[Sort Calibration]: Displays the [Auto Calibration] wizard for calibrating sorting.

For details, see "Automatic Calibration" (page 46).

[Chip Alignment]: Displays the [Chip Alignment] screen of the [Auto Calibration] wizard for aligning the sorting chip.
Follow the on-screen instructions to align the sorting chip. During automatic alignment, the progress bar and status are displayed, allowing you to check the progress and current state.

[Temperature Control] group

Contains tool buttons for controlling the temperature of the collection stage and sample loader to maintain sample fluid temperature.
The following temperature control options are available.
- Collection stage: 5 °C (41 °F) only
- Sample loader: 5 °C (41 °F) or 37 °C (98.6 °F)

[Collection]: Turns the collection temperature control on/off and displays the current temperature setting.
[Sample]: Turns the sample loader temperature control on/off and displays the current temperature setting.
Clicking the dialog launcher displays the [Temperature Control Settings] dialog *(page 120)* for specifying the temperature settings.

[Agitate] group

Contains a tool button for controlling agitation of the sample fluid.

[Agitate]: Turns the sample loader agitation unit on/off.

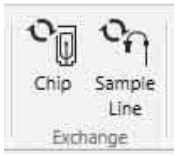
[Safety] group

Contains a tool button for running a suction fan for safety.

[Vacuum Fan]: Displays the [Vacuum Fan Control] dialog *(page 121)* for setting a timer for operation of the vacuum fan.
The fan is used to extract aerosols (microparticles of biological and other matter in suspension created by sorting) from the sorting area. Setting the duration to "0" runs the fan without the timer function.

[Exchange] group

Contains a tool button for exchanging the sorting chip.



[Chip]: Ejects the sorting chip and displays the [Chip Exchange] wizard for inserting a different sorting chip.

For details, see "Exchanging the Sorting Chip" (page 67).

[Sample Line]: Displays the [Sample Line Exchange] wizard for changing the sample line.

For details, see "Changing the PEEK Sample Line (PEEK sample line compatible models)" (page 169) or "Changing the Sample Line and Probe (Non-PEEK sample line compatible models)" (page 172).

Tip

The sorting chip must be replaced or reinserted after changing the sample line.

[Settings] group

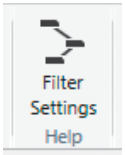
Contains tools button for configuring advanced settings related to the instrument.

[Settings]: Displays the [Cytometer Settings] dialog *(page 121)* for configuring the instrument, adjusting sort parameters, and other instrument functions.

[Droplet]: Displays a detailed image from the droplet camera.

[Help] group

Contains a tool button for displaying the optical filter pattern.



[Filter Settings]: Displays the configured optical filter pattern. This cannot be modified.

[Shutdown] group

Contains tool buttons used when shutting down the SH800.

[Hardware Only]: Displays the [Shutdown Wizard] screen for cleaning the SH800 and then shutting down the instrument only.

For details, see "Shutdown" (page 65).

[Software and Hardware]: Displays the [Shutdown Wizard] screen for cleaning the SH800 shutting down the instrument and SH800 Software.

For details, see "Shutdown" (page 65).

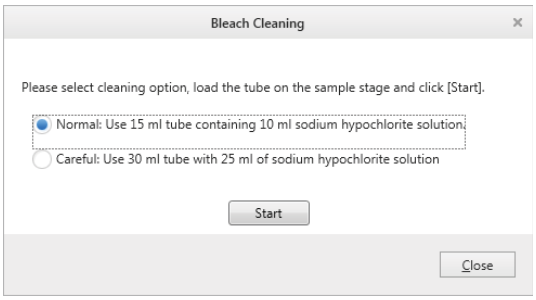
[Ethanol Cleaning]: Displays the Ethanol Cleaning wizard for cleaning the internal sheath fluid line and DI water line with ethanol. The SH800 instrument and SH800 Software automatically shut down after cleaning is finished.

For details, see "Cleaning the Internal Sheath Line and DI Water Line using Ethanol" (page 188).

[Bleach Cleaning] dialog

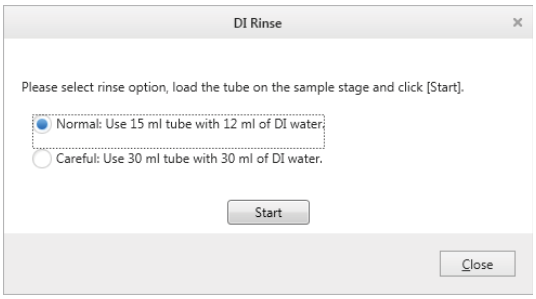
The [Bleach Cleaning] dialog is displayed by clicking [Bleach Cleaning] in the [Cleaning] group on the [Cytometer] tab of the ribbon.
The [Bleach Cleaning] dialog is used to start cleaning of the sorting chip, sample line, and sample probe using a Sodium Hypochlorite (bleach) solution.

For details, see "Cleaning the Sample Fluidics System using Bleach" (page 152).


Bleach Cleaning
Please select cleaning option, load the tube on the sample stage and click [Start].
Normal: Use 15 ml tube containing 10 ml sodium hypochlorite solution.
Careful: Use 30 ml tube with 25 ml of sodium hypochlorite solution
Start
Close

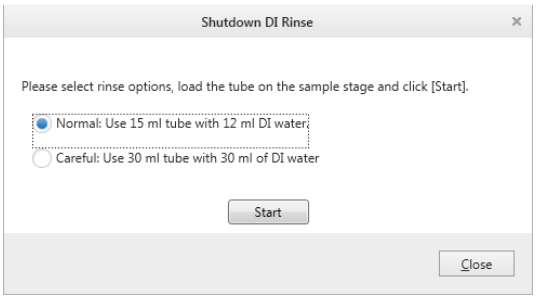
[DI Rinse] dialog

The [DI Rinse] dialog is displayed by clicking [DI Rinse] in the [Cleaning] group on the [Cytometer] tab of the ribbon.
The [DI Rinse] dialog is used to start flushing of the sorting chip, sample line, and sample probe using DI water.
Select the size of conical tube containing DI water to use, then click [Start] to load the tube and begin cleaning.


DI Rinse
Please select rinse option, load the tube on the sample stage and click [Start].
Normal: Use 15 ml tube with 12 ml of DI water.
Careful: Use 30 ml tube with 30 ml of DI water.
Start
Close

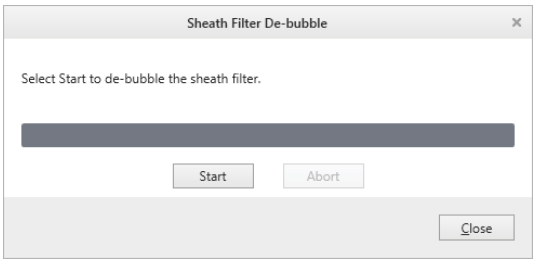
[Shutdown DI Rinse] dialog

The [Shutdown DI Rinse] dialog is displayed by clicking [Shutdown Rinse] in the [Cleaning] group on the [Cytometer] tab of the ribbon.
The [Shutdown DI Rinse] dialog is used to clean the sorting chip, sample line, and sample probe using DI water, and then automatically shutting down.
Select the size of conical tube containing DI water to use, then click [Start] to load the tube and begin cleaning.


Shutdown DI Rinse
Please select rinse options, load the tube on the sample stage and click [Start].
Normal: Use 15 ml tube with 12 ml DI water.
Careful: Use 30 ml tube with 30 ml of DI water
Start
Close

[Sheath Filter De-bubble] dialog

The [Sheath Filter De-bubble] dialog is displayed by clicking [Sheath Filter] in the [De-bubble] group on the [Cytometer] tab of the ribbon.
The [Sheath Filter De-bubble] dialog is used to remove air bubbles from the sheath filter. Click [Start] to begin the process.

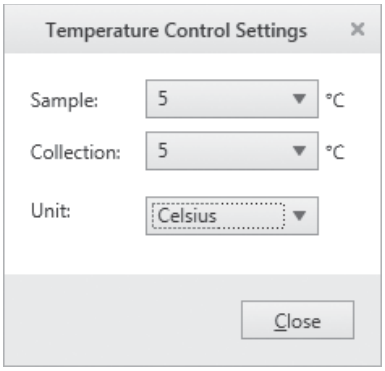


Tip

The sheath filter de-bubble function should be run at least three times after changing the sheath filter.

[Temperature Control Settings] dialog

The [Temperature Control Settings] dialog is displayed by clicking the dialog launcher in the [Temperature Control] group on the [Cytometer] tab of the ribbon.
The [Temperature Control Settings] dialog is used to set the temperature of the sample loader and collection stage area.



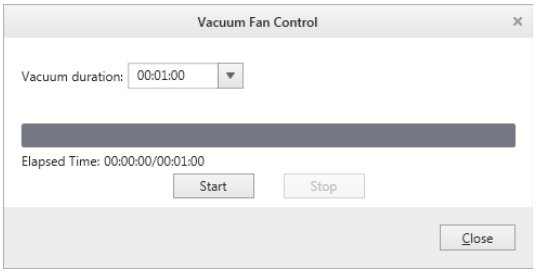
[Sample]: Sets the control temperature of the sample loader to 5 °C (41 °F) or 37 °C (98.6 °F).
The selected temperature values are displayed on the corresponding buttons in the [Temperature Control] group on the ribbon.

[Collection]: Selects the control temperature of the collection stage area. Only 5 °C (41 °F) is available.

[Unit]: Selects the units of temperature (Celsius or Fahrenheit).
[Close]: Closes the dialog.

[Vacuum Fan Control] dialog

The [Vacuum Fan Control] dialog is displayed by clicking [Vacuum Fan] in the [Safety] group on the [Cytometer] tab of the ribbon.
The [Vacuum Fan Control] dialog is used to set a timer for operation of the vacuum fan.
The fan is used to extract aerosols (microparticles of biological and other matter in suspension created by sorting) from the sorting area.

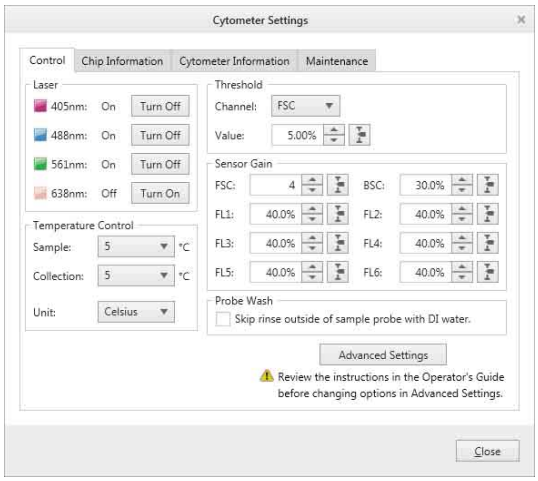


[Vacuum duration]: Set the duration for fan operation. Setting the duration to "00:00:00" allows you to operate the fan for an unlimited duration. When you want to stop the fan in such cases, click [Stop].
[Elapsed Time]: Displays the elapsed time since starting the fan.
[Start]: Starts the fan.
[Stop]: Stops the fan.
[Close]: Closes the dialog.

[Cytometer Settings] dialog

The [Cytometer Status] dialog is displayed by clicking [Settings] in the [Settings] group on the [Cytometer] tab of the ribbon.
The [Cytometer Settings] dialog is used to configure instrument settings, adjust sort parameters, and other functions.

- **[Control] tab**
  This tab is used to configure instrument settings.



[Laser]: Turns each laser on/off.
[Temperature Control]: Sets the control temperature of the sample loader and collection stage.

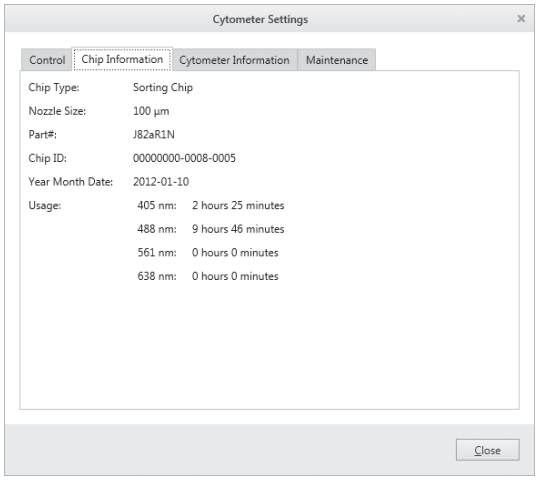
For details about each item, see "[Temperature Control Settings] dialog" (page 120).

[Threshold]: Sets the threshold trigger for detecting events.
- [Channel]: Selects the channel (FSC, BSC, FL1 to FL6) used to trigger the detection of events.
- [Value]: Sets the input threshold signal level of the trigger channel as a percentage of the maximum signal from the detection module.

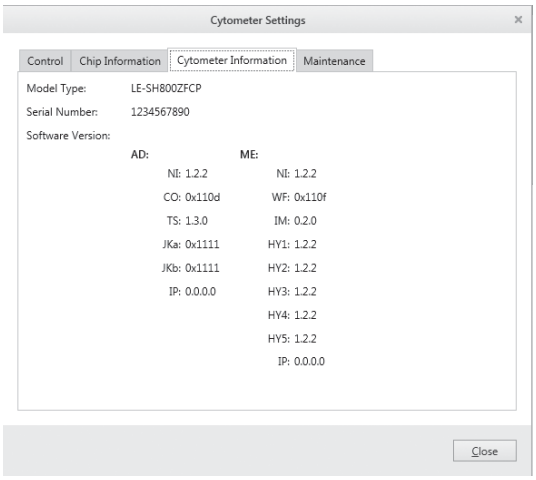
[Sensor Gain]: Sets the gain of each detector.
- [FSC]: Selects the gain of the forward-scatter detector. The gain setting has a 16-step range.
- BSC, FL1 to FL6: Sets the gain of the corresponding back scatter and fluorescence photomultiplier tube (PMT) detectors as a percentage of the maximum level.

[Probe Wash]: Sets probe wash options. To skip cleaning of the outer surface of the sample probe using DI water from the DI water tank when cleaning the probe, place a check mark in the [Skip rinse outside of sample probe with DI water.] checkbox. If a check mark is placed in this checkbox, you should load a sample tube containing DI water, after cleaning the sample probe, to clean the outside of the probe.
[Advanced Settings]: Displays the [Advanced Settings] dialog *(page 122)* for manually adjusting sort parameters.

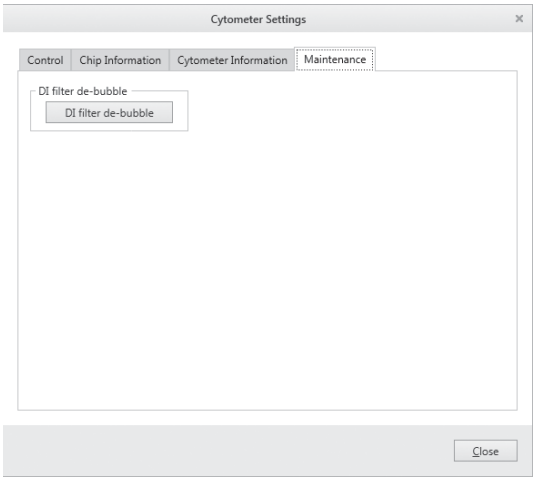
- **[Chip Information] tab**
  Displays information about the loaded sorting chip. This is useful for checking the length of time the sorting chip has been in use.



- **[Cytometer Information] tab**
  Displays SH800 instrument information.



- **[Maintenance] tab**
  Contains a function to remove air bubbles from the DI water filter.
  Click [DI Filter de-bubble] to display the [DI Filter de-bubble] dialog and then click [Start].

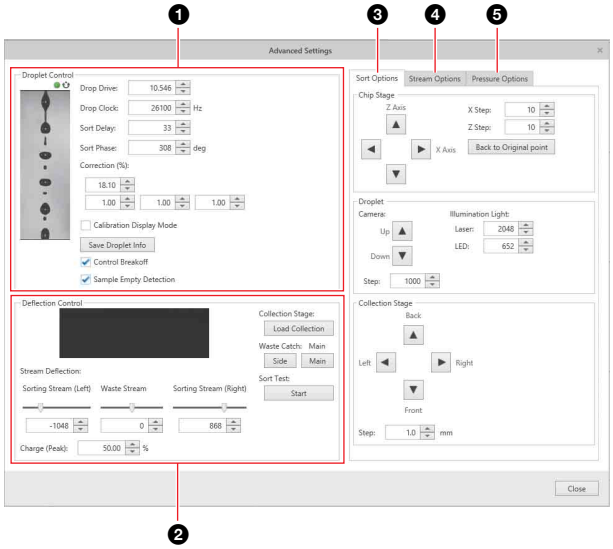


Tip

The DI water filter de-bubble function should be run at least three times after changing the DI water filter.

For details, see "Changing the DI Water Filter" (page 177).

- **[Advanced Settings] dialog**
  The [Advanced Settings] dialog is used to adjust sort parameters manually.



❶ [Droplet Control]
Contains adjustments related to droplet formation.

[Drop Drive]
The drop drive setting adjusts the amplitude of the piezoelectric element drive voltage. The frequency sets the cycle time of vibration of the sorting chip. The vibration of the sorting chip causes the stream to break into droplets after the stream is emitted from the chip nozzle.
The drop drive is optimized during auto calibration.

[Drop Clock]
The drop clock setting adjusts the frequency of the vibration of the piezoelectric element drive voltage. The frequency sets the cycle time of vibration of the sorting chip.
The drop drive is optimized during auto calibration.

[Sort Delay]
The sort delay setting adjusts the number of vibrations applied to the sorting chip as cells pass between the optical detection point and the breakoff point. It is used to determine the timing of the electrostatic charging pulse applied to the sheath fluid.
The sort delay is optimized during auto calibration.

[Sort Phase]
The sort phase setting adjusts the phase difference of the charging pulse waveform as cells pass between the optical detection point and the breakoff point. It is used to determine the timing of the electrostatic charging pulse applied to the sheath fluid.

The sort phase is optimized during auto calibration.

[Correction (%)]

The correction setting adjusts the shape of the charging waveform applied to successive droplets.

- Top spinbox: Controls the overall charging waveform correction adjustment.
- Lower spinboxes: Fine-tunes the correction applied to the first droplet, second droplet, and third droplet, respectively.

When droplets are electrostatically charged, the next droplet receives an induced charge of the opposite polarity, which can affect the angle of deflection as the droplets pass through the deflection plates (a problem called fanning). The correction function uses a defanning algorithm to adjust the charging waveform applied to successive droplets to reduce fanning.

[Calibration Display Mode]

The calibration display mode changes the image displayed by the droplet camera so that only droplets containing automatic setup beads are displayed.
This mode is used for fine manual control of the sort delay, sort phase, and related sort parameters.

[Save Droplet Info]

Saves the current droplet image as a reference image used by the Control Breakoff function. Clicking this button displays a confirmation message. Click [Yes] to save the current droplet image.

[Control Breakoff]

The control breakoff setting uses a feedback system to control the breakoff position automatically.
When this function is enabled, the drop drive is adjusted so as to maintain the optimized breakoff point and side streams obtained during auto calibration.
When auto calibration finishes, the Control Breakoff function is automatically enabled.

Tips

- The drop drive and drop clock cannot be adjusted manually while the Control Breakoff function is enabled.
- When breakoff feedback control is enabled, if the breakoff point moves and cannot be recovered within a predetermined time, sorting stops and an error message is displayed on the screen.

[Sample Empty Detection]

The empty detection function automatically stops data acquisition when the sample tube becomes empty. The function detects disturbances in the droplet stream as the sample fluid in the sample tube is used up, and then stops data acquisition.

Tips

- The sample empty detection function may stop data acquisition if cells of size 20 µm or larger are in the sample. If this occurs, disable the sample empty function to continue data acquisition.
- If a sample empty state is detected, click [Chip] in the [De-bubble] group on the [Cytometer] tab of the ribbon in SH800 Software, and repeatedly de-bubble the sorting chip until the breakoff point returns to its original position.

❷ [Deflection Control]

Contains controls for adjusting the side stream deflection angle, loading/unloading the collection stage, and running a sorting test.

[Stream Deflection]

Adjusts the angle of deflection of the two side streams.

[Charge (Peak)]

Sets the peak value of the charging voltage waveform.

[Collection Stage]

Loads/unloads the collection stage.

[Waste Catch]

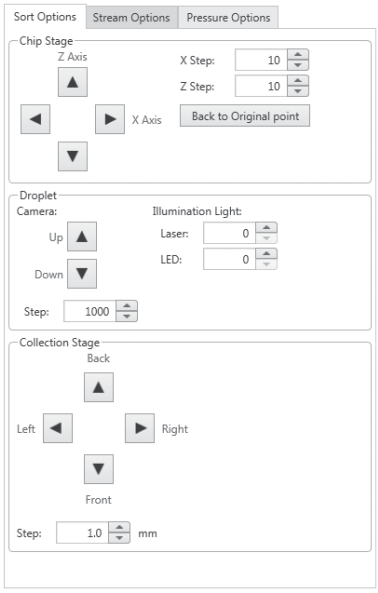
Moves the sorting area waste catcher to the side.

[Sort Test]

Runs a sorting test.

❸ [Sort Options] tab

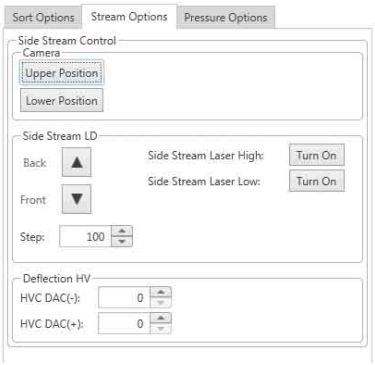
The sort option settings are used to adjust the position of the sorting chip, droplet camera, and collection stage manually.



[Chip Stage]: Adjusts the positioning of the sorting chip within the chip loader.
[Droplet]: Adjusts the vertical positioning of the droplet camera and the brightness of the droplet camera laser and backlight.
[Collection Stage]: Adjusts the horizontal position of the collection stage.

❹ [Stream Options] tab

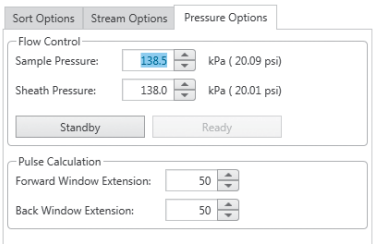
Adjusts side stream settings.



[Camera]: Sets the viewing position (upper or lower) of the side stream monitor.
[Side Stream LD]: Adjusts the horizontal position and power of the side stream monitor laser.
[Deflection HV]: Adjusts the voltage applied to each of the deflection plates.

❺ [Pressure Options] tab

Sets the sample fluid flow controls and the window extension settings.



[Flow Control]: Adjusts the sample pressure and sheath pressure.
Clicking [Standby] stops the sheath and sample fluid flow. Clicking [Ready] starts sheath flow again in preparation for a sample.
[Pulse Calculation]: Sets the forward window extension (FWE) and back window extension (BWE) settings used to modify the pulse parameters for data acquisition.

Tip

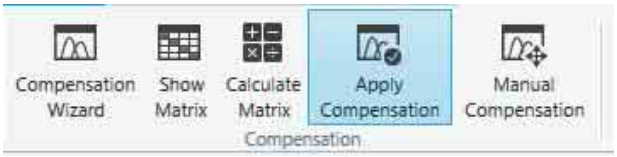
The setting of the forward window extension and back window extension affect the data acquisition results for Area pulse parameters.

[Compensation] Tab (ribbon)

The [Compensation] tab of the ribbon has the following buttons.

[Compensation] group

Contains tool buttons related to fluorescence compensation.



[Compensation Wizard]: Displays the [Compensation Wizard] for recording control tubes in a compensation panel.

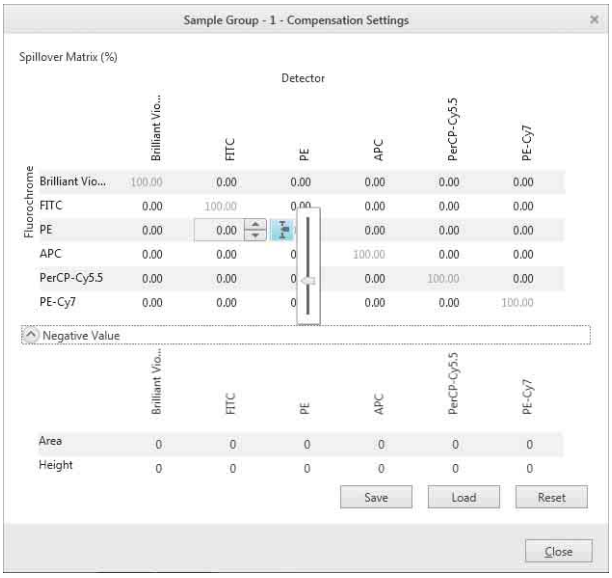
The compensation settings should normally be configured using the [Compensation Wizard] to record a negative (unstained) control tube and up to six positive (stained) control tubes in a compensation panel. The [Compensation Wizard] then automatically calculates the fluorescence compensation spillover matrix for the sample group.

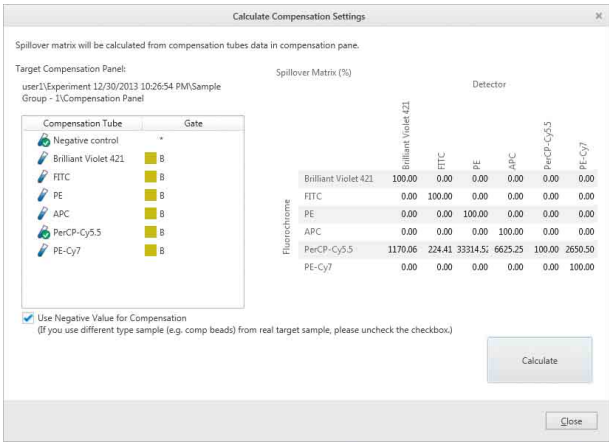
For details, see "Performing Fluorescence Compensation" (page 61).

[Show Matrix]: Displays the [Compensation Settings] dialog *(page 124)* for viewing the spillover matrix, and loading/saving the spillover matrix.
[Calculate Matrix]: Displays the [Calculate Compensation Settings] dialog *(page 125)* for calculating the coefficients of the fluorescence compensation spillover matrix based on the negative control tubes and the compensation panel.
[Apply compensation]: Selects whether fluorescence compensation is applied to plots on the worksheet. Clicking this button toggles compensation on/off.
[Manual Compensation]: Toggles manual fluorescence compensation mode on/off for the plots on the worksheet.

[Compensation Settings] dialog

The [Compensation Settings] dialog is displayed by clicking [Show Matrix] on the [Compensation] tab of the ribbon.
The [Compensation Settings] dialog is used to view the spillover matrix, and load/save the spillover matrix.





Tip

Matrix coefficients cannot be added or deleted.

[Spillover Matrix (%)]: Displays the fluorescence compensation spillover matrix.
Spillover matrix values display the fluorescence intensity due to each fluorochrome as a percentage of the total intensity in each channel.
Spillover matrix coefficients can be modified manually.

Note

Changing compensation values directly is only recommended for experienced users.

[Negative Value]: Displays the spillover matrix for the negative control tube for both area and height pulse parameters.
[Save]: Saves the compensation settings as an XML file. You can load saved compensation settings into another sample group or another experiment.
[Load]: Loads previously saved compensation settings from an XML file.
[Reset]: Resets all compensation settings.
[Close]: Closes the dialog.

[Calculate Compensation Settings] dialog

The [Calculate Compensation Settings] dialog is displayed by clicking [Calculate Matrix] on the [Compensation] tab of the ribbon.
The [Calculate Compensation Settings] dialog is used to calculate the coefficients of the fluorescence compensation spillover matrix based on the negative control tubes and the compensation panel.

[Target Compensation Panel]: Displays the compensation panel for which to calculate fluorescence compensation.
[Use Negative Value for Compensation]: Enables calculation of the spillover matrix using a negative control. If an appropriate negative control is not available, deselect the checkbox to calculate the spillover matrix without using a negative control. The checkbox default setting is on (enabled).
[Spillover Matrix (%)]: Displays the spillover matrix. Spillover matrix values display the fluorescence intensity due to each fluorochrome as a percentage of the total intensity in each channel.
[Calculate]: Calculates the spillover matrix coefficients.
[Close]: Closes the dialog.

[Worksheet Tools] Tab (ribbon)

The [Worksheet Tools] tab of the ribbon has the following buttons.

[Plot] group

Contains tool buttons for adding, deleting, and duplicating plots.

For details, see "Adding a Plot" (page 83).

[New Density]: Adds a new density plot to the worksheet.
[New Dot Plot]: Adds a new dot plot to the worksheet.
[New Histogram]: Adds a new histogram plot to the worksheet.
[Remove Plot]: Removes the selected plot from the worksheet.
[Duplicates Plot]: Duplicates the selected plot on the worksheet.

[Display Events] group

Contains tool buttons for setting the number of events displayed on plots.

[Acquisition]: Sets the number of events to display on plot during data acquisition.
[Analysis]: Sets the number of events to display on plot when analyzing data.

[Gate] group

Contains tool buttons for editing gates.

For details, see "Editing Gates" (page 91).

[Remove Gate]: Deletes the selected gate.

Note

Deleting a gate will also delete all gates and plots that are derived from the selected gate.

[Edit Gate]: Displays the [Gate Editor] dialog *(page 127)* for editing gates.

[Statistics] group

Contains tool buttons for working with statistics.

For details, see "Displaying Statistics" (page 92).

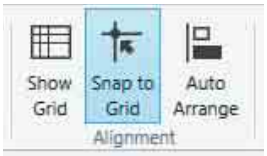
[Show Table]: Shows/hides the [Gates and Statistics] table.
[Edit Statistics]: Displays the [Statistics Editor] dialog for configuring the attributes displayed in the [Gates and Statistics] table.

For details about the [Statistics Editor] dialog, see "Displaying Statistics" (page 92).

[Export Statistics to CSV File]: Displays the [Exporting statistics table] dialog *(page 128)* for exporting the data in the [Gates and Statistics] table as a CSV-format file.

[Alignment] group

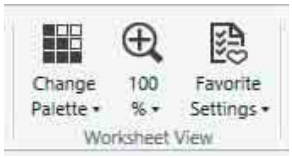
Contains tool buttons for displaying a grid on the worksheet and for aligning displayed items with the grid.



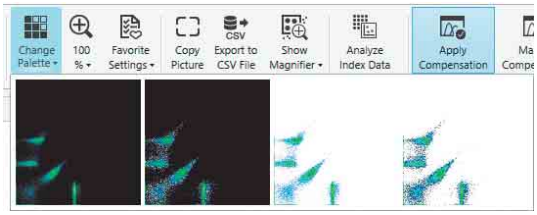
[Show Grid]: Displays the grid on the worksheet.
[Snap to Grid]: Aligns the plots and [Gates and Statistics] table on the worksheet with the grid when they are moved or added.
[Auto Arrange]: Automatically aligns the plots and [Gates and Statistics] table on the worksheet to the grid.

[Worksheet View] group

Contains tool buttons related to the display of items on the worksheet, and for saving the current settings.



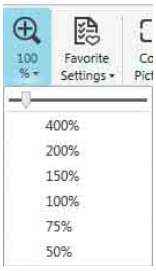
[Change Palette]: Displays a drop-down palette for changing the display style of the selected plot.



- Black background
- Black background with highlighted outlying events
- White background
- White background with highlighted outlying events

Highlighting outlying events is useful for identifying and analyzing rare events.

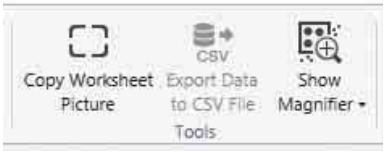
Zoom%: Displays a drop-down menu for changing the display zoom factor of the worksheet.
The current zoom factor is displayed on the button icon.
This setting is applied to all plots.
Click this button and select the zoom factor from the menu, or use the slider to set the zoom factor.



[Favorite Settings]: Allows you to store favorite worksheet settings.
- [Save as Favorite]: Saves the current worksheet state as the default settings.
- [Restore from Favorite]: Applies saved settings to the current worksheet.

[Tools] group

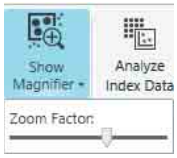
Contains tool buttons for copying, exporting, and displaying data on the worksheet.



[Copy Worksheet Picture]: Copies the selected worksheet to the clipboard for pasting in other software, for example to produce reports.

[Export Data to CSV File]: Exports the recorded data for the sample tube as a CSV file.
Clicking this button displays the [Exporting Data] dialog *(page 128)*.
If conducting index sorting, you can export only the index sorted data by selecting [Well Select Mode] in the [Index Analysis] dialog *(page 102)*.

[Show Magnifier]: Shows/hides a worksheet magnifier.
You can also set the zoom factor.



[Index Sorting] group

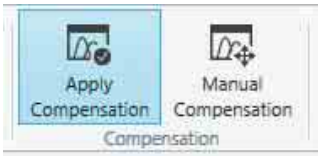
Contains a command button for analyzing data using the index sorting function.

[Analyze Index Data]: Displays the [Index Analysis] dialog for emphasizing the position on plots of cells sorted in each well in multi-well plates.

For details about the [Index Analysis] dialog, see "Analyzing Index Sort Data" (page 102).

[Compensation] group

Contains tool buttons related to fluorescence compensation.

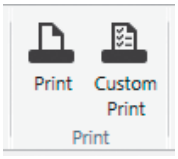


[Apply compensation]: Selects whether fluorescence compensation is applied to plots on the worksheet. Clicking this button toggles compensation on/off.

[Manual Compensation]: Toggles manual fluorescence compensation mode on/off for the plots on the worksheet.

[Print] group

Contains tool buttons for printing statistics and plots displayed on the worksheet.



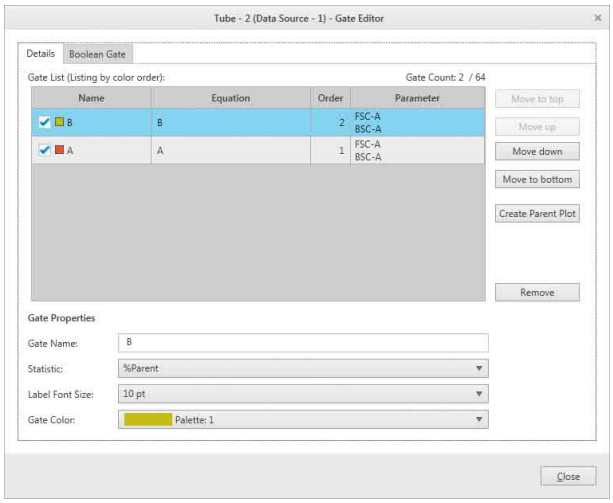
[Print]: Displays the [Print] window *(page 108)* for printing the worksheet.

[Custom Print]: Displays the [Custom Print] dialog *(page 128)* for selecting the items to print.

[Gate Editor] dialog

The [Gate Editor] dialog is displayed by clicking [Edit Gate] on the [Worksheet Tools] tab, [Plot Tools] tab, or [Gate Tools] tab of the ribbon.
The [Gate Editor] dialog is used to edit gates.

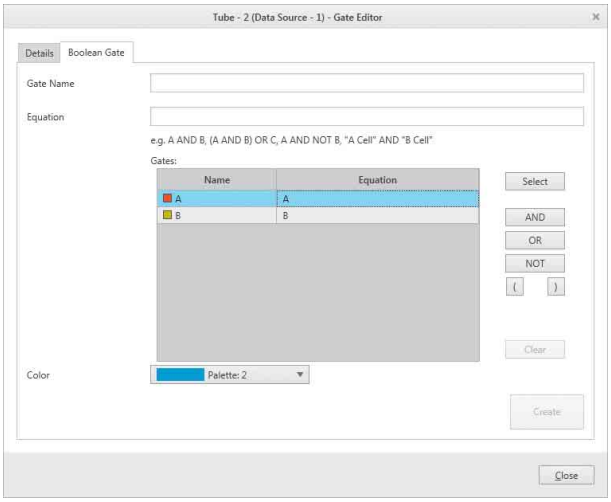
- **[Details] tab**
  Edits attributes of gates.



[Gate List]: Displays a list of the configured gates. You can show or hide a gate on plots using the checkbox beside the gate name.
[Move to top]: Moves the selected gate to the top of the gate list, and displays the selected color at the front on dot plots.
[Move up]: Moves the selected gate up one row in the gate list, and displays events with the selected color one rank towards the front on dot plots.
[Move down]: Moves the selected gate up one down in the gate list, and displays events with the selected color one rank towards the back on dot plots.
[Move to bottom]: Moves the selected gate to the bottom of the gate list, and displays the selected color at the back on dot plots.
[Create Parent Plot]: Adds a parent plot to the worksheet.
[Remove]: Deletes the selected gate.
[Close]: Closes the dialog.

- **[Boolean Gate] tab**
  Adds a Boolean gate to the gate hierarchy.

  For details about the [Boolean Gate] tab, see "Adding a Boolean gate to the Gate Hierarchy" (page 89).



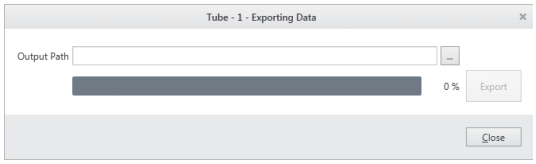
[Exporting statistics table] dialog

The [Exporting statistics table] dialog is displayed by clicking [Export Statistics to CSV File] on the [Worksheet] tab or [Plot Tools] tab of the ribbon.
The [Exporting statistics table] dialog is used to export the contents of the [Gates and Statistics] table as a CSV-format file.

[Output Path]: Specifies the export destination folder. Click ... (Browse) to specify for a destination folder.
Progress bar: Displays the export progress.
[Export]: Starts the export.
[Close]: Closes the dialog.

[Exporting Data] dialog

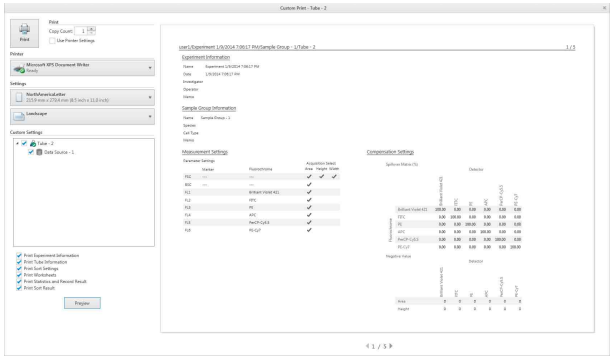
The [Exporting Data] dialog is displayed by clicking [Export Data to CSV File] on the [Worksheet] tab of the ribbon.
The [Exporting Data] dialog is used to export recorded data for sample tubes as a CSV-format file.



[Output Path]: Specifies the export destination folder. Click ... (Browse) to specify for a destination folder.
Progress bar: Displays the export progress.
[Export]: Starts the export.
[Close]: Closes the dialog.

[Custom Print] dialog

The [Custom Print] dialog is displayed by clicking [Custom Print] in the [Print] group on the [Worksheet Tools] tab of the ribbon.
The [Custom Print] dialog is used to select items for printing.



[Custom Settings]: Selects the sample tubes for printing. Select the checkbox for a tube to print the worksheet for the tube.
[Print Experiment Information]: Selects whether to print the experiment information settings.
[Print Tube Information]: Selects whether to print the sample tube information settings.
[Print Sort Settings]: Selects print of the sort settings.
[Print Worksheets]: Selects printing of the worksheet settings.
[Print Statistics and Record Result]: Selects printing of the [Gates and Statistics] table and recorded results.
[Print Sort Result]: Selects print of the sorting results.
[Preview]: Displays a print preview image on the right side of the screen.

For details about other items, see "[Print] Window" (page 108).

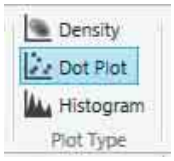
[Plot Tools] Tab (ribbon)

The [Plot Tools] tab of the ribbon has the following buttons.

Refer to the [Worksheet Tools] tab for a description of the [Plot] group buttons (page 125), [Statistics] group buttons (page 126), [Tools] group buttons (page 127), and [Compensation] group buttons (page 127).

[Plot Type] group

Contains tool buttons for changing the type of the selected plot.



[Scale Type] group

Changes the type of scale displayed on axes on plots.

[X Axis] / [Y Axis]: Changes the type of scale displayed on the X axis and Y axis, respectively, of the selected plot.

For details about axis scale types, see "Changing Axis Scales" (page 85).

[Swap]: Swaps the position of the X axis and Y axis scales.

: Displays the [Property Window] dialog *(page 130)* for specifying axis types and scale ranges.

[Axes] group

Contains tool buttons for adjusting axis scales.

[Auto Adjust (XY)]: Automatically adjusts the ranges of the X axis and Y axis of the selected plot to their minimum and maximum measured values.

[Auto Adjust (X Axis)] / [Auto Adjust (Y Axis)]: Automatically adjusts the range of the X axis or Y axis, respectively, of the selected plot to the minimum and maximum measured values.

[Zoom]: Zooms in on the area selected by dragging the mouse on a plot.

[Default Adjust]: Restores the default X axis and Y axis ranges. Click the down arrow to set the number of decades displayed on log scales.

: Displays the [Property Window] dialog *(page 130)* for specifying axis types and scale ranges.

[Gate] group

Contains tool buttons for drawing gates on plots and editing gate properties.

For details, see "Adding Gates" (page 88) and "Editing Gates" (page 91).

[Rectangle]: Creates a rectangular gate on a dot plot or density plot.

[Ellipse]: Creates an elliptical gate on a dot plot or density plot.

[Polygon]: Creates a polygonal gate on a dot plot or density plot.

[Quadrant]: Creates a quadrant gate on a dot plot or density plot.

[Linear]: Creates a linear gate on a histogram plot.

[Remove Gate]: Deletes the selected gate.

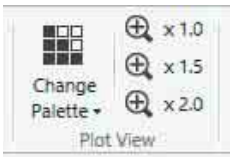
Note

Deleting a gate will also delete all gates and plots that are derived from the selected gate.

[Edit Gate]: Displays the [Gate Editor] dialog *(page 127)* for editing gates.

[Plot View] group

Contains tool buttons for changing the display of plots.



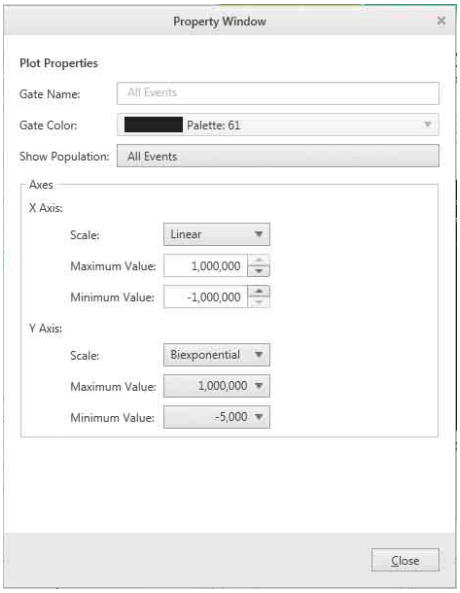
[Change Palette]: Displays a drop-down palette for changing the display style of the selected plot.

For details, see "[Change Palette]" (page 126).

x1.0, x1.5, x2.0: Displays the plot at 100% (default), 150%, and 200% size, respectively.

[Property Window] dialog

The [Property Window] dialog is displayed by clicking the dialog launcher in the [Scale Type] group or [Axes] group on the [Plot Tools] tab of the ribbon. You can also display the dialog by right-clicking the worksheet, plot, gate, or a [Gates and Statistics] table cell, and selecting [Properties] from the context menu. The [Property Window] dialog is used to change the attributes of plots, and to specify the scale type and range of axes.



[Gate Name]: Displays the name of the gate (required). You can edit the name, as required. Note that the names of gates must be unique.
[Gate Color]: Selects the color of the gate on plots.
[Show Population]: Selects the population to display on plots.
[Axes]: Specifies the scale type and range of the X and Y axes.
- [Scale]: Selects the scale type.
- [Maximum Value]: Specifies the maximum value of the scale.
- [Minimum Value]: Specifies the minimum value of the scale.

[Close]: Closes the dialog.

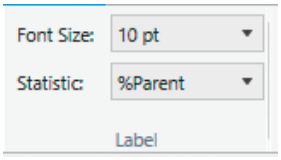
[Gate Tools] Tab (ribbon)

The [Gate Tools] tab of the ribbon has the following buttons.

Refer to the [Worksheet Tools] tab for a description of the [Gate] group buttons (page 126).

[Label] group

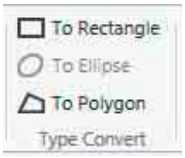
Contains tool buttons for specifying gate labels.



[Font Size]: Selects the font size of text labels.
[Statistic]: Selects the statistic that is displayed beside gates on plots.

[Type Convert] group

Contains tool buttons for converting the selected gate to a different gate type.

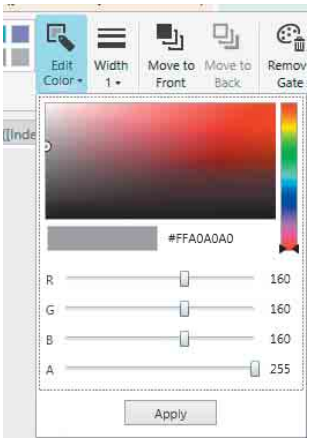


[Style] group

Contains tool buttons for changing the display style of gates.

[Visible]: Shows/hides the selected gate.
Color palette: Changes the color of the selected gate.

[Edit Color]: Allows you to edit the color in the lower color palette (user defined colors) to any color.
Click a color in the color palette, click [Edit Color], then select a new color and click [Apply].



[Width]: Selects the line width of the selected gate.

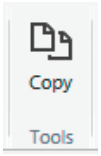
[Color Order] group

Contains tool buttons for changing the display order of gates.

[Move to Front]: Moves the selected gate to the front.
[Move to Back]: Moves the selected gate to the back.

[Tools] group

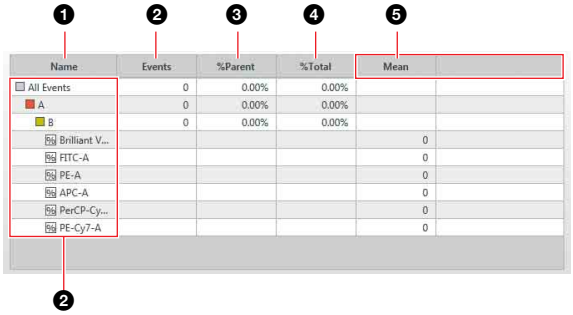
Contains a tool button for copying gates.



[Copy]: Copies the selected gate.

[Gates and Statistics] Table

The [Gates and Statistics] table displays the following information.



❶ [Name]
Displays the names of gates and parameters.

❷ [Events]
Displays the number of events.

❸ [%Parent]
Displays the proportion of events of each gate relative to its parent.

❹ [%Total]
Displays the proportion of events of each gate relative to the total of all events.

❺ Other statistics values
Displays the values of statistics configured in the [Statistics Editor] dialog.

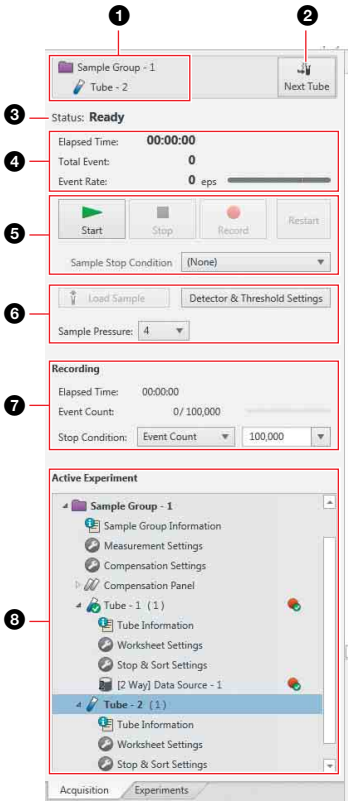
- [Mean]: Average value of events in population
- [Minimum]: Minimum value in a population
- [Maximum]: Maximum value in a population
- [SD]: Standard deviation of events from the mean value in a population
- [CV]: Coefficient of variation of events in a population
- [Median]: Median value (equal number of events above and below)
- [Mode]: Value with highest event count (corresponds to the highest peak on a histogram plot)
- [HPCV]: Half-peak coefficient of variation (coefficient of variation from width of distribution at half peak height)

For details, see "Displaying Statistics" (page 92).

❻ Gate hierarchy
Displays the hierarchical relationship between gates. The color to the left of the gate name shows the color of the gates on plots.

[Acquisition] Tab (control pane)

The [Acquisition] tab controls data acquisition and recording.



❶ Currently assigned sample tube and sample group

❷ [Next Tube]

Assigns the next tube.
If there are no further tubes, it duplicates the current tube and assigns the new tube.

❸ Instrument status

Displays the status of the instrument.
The following states are displayed.

- Initializing
- Standby
- Ready
- Pause
- Measurement
- Probe Wash
- No Chip
- Chip De-bubble
- Ethanol Cleaning
- Error
- Waiting
- Bleach Cleaning
- DI Rinse
- Shutdown

❹ Data acquisition status

Displays the status of data acquisition.

[Elapsed Time]: Displays the cumulative elapsed time since the start of data acquisition.

[Total Events]: Displays the total number of events detected during data acquisition.

[Event Rate]: Displays the rate events are detected during data acquisition in events per second (eps).

Range: 0 eps to 20,000 eps.

The event rate indicator on the right hand side shows the current event rate relative to the guaranteed performance rate of 10,000 eps.

Tip

It is recommended that the event range be reduced if the indicator exceeds a rate of 10k.

❺ Data acquisition controls

(Start): Starts/resumes data acquisition.
It does not start data recording.

(Pause): Pauses data acquisition.
The elapsed time continues counting while acquisition is paused.

(Stop): Stops data acquisition manually.

(Record): Records the acquired data.

(REC stop): Stops data recording manually.

(Restart): Resets the total events and elapsed time counters to 0 and restarts data acquisition.

Tip

If an automatic stop condition has been specified, data acquisition and/or recording stops automatically when the corresponding stop condition has been satisfied.

[Sample Stop Condition]: Selects the stop condition for stopping data acquisition automatically.

- None (data acquisition will stop automatically when the number of detected events exceeds 100,000,000 or the elapsed time exceeds 12:00:00, whichever occurs first)
- Recording (stops data acquisition automatically when the recording stop condition is satisfied)
- Sorting (stops data acquisition automatically when the sorting stop condition is satisfied)
- Recording and Sorting (stops data acquisition automatically when both the recording and sorting stop conditions are satisfied)

❻ Instrument controls

[Load Sample] / [Unload Sample]: Loads/unloads the sample tube.
These operations are enabled only when data acquisition is paused.

[Detector & Threshold Settings]: Displays the [Detector & Threshold Settings] dialog.

For details about the [Detector & Threshold Settings] dialog, see "Configuring Detector Settings" (page 79).

[Sample Pressure]: Sets the sample fluid pressure (range of 1 to 10).

❼ Recording controls

[Elapsed Time]: Displays the cumulative elapsed time since the start of data recording.

[Event Count]: Displays the number of recorded events.

[Stop Condition]: Selects the stop condition for stopping data recording automatically.

- None
- Elapsed Time
  Specifies elapsed time condition to stop recording automatically.
- Event Count
  Specifies the event count condition to stop recording automatically.

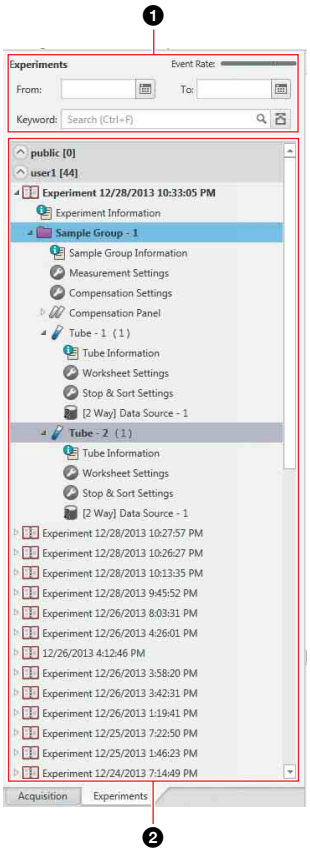
❽ Experiment Explorer (Active Experiment)

Displays the structure of the active experiment enabled for data acquisition, recording, and cell sorting.

For details about the Experiment Explorer, see "Experiment Explorer" (page 133) on the [Experiments] tab (control pane).

[Experiments] Tab (control pane)

The [Experiments] tab displays all current public (shared) and private experiments.



❶ Search criteria

You can search experiments by date and keyword.

The search results are displayed in the Experiment Explorer.

For details about searching, see "Searching Experiments" (page 77).

❷ Experiment Explorer

Displays a list of all current experiments.
The internal structure of each experiment is displayed in a hierarchical list view.

[public]: Lists shared experiments. Shared experiments can be viewed by all users.

User_name: Lists private experiments created by the logged-in user. Private experiments cannot be viewed by other users.

An experiment contains the following structural components.

Experiment

[Experiment Information]: Contains information about the experiment. Double-clicking this component displays the [Experiment Information] dialog *(page 134)*.

Sample group: Displays the name of a sample group. A sample group contains the following components.

[Sample Group Information]: Contains information about the sample group. Double-clicking this component displays the [Sample Group Information] dialog *(page 134)*.

[Measurement Settings]: Contains instrument settings. Double-clicking this component displays the [Measurement Settings] dialog *(page 73)*.

[Compensation Settings]: Contains the fluorescence compensation settings for the fluorochromes used. Double-clicking this component displays the [Compensation Settings] dialog *(page 124)*.

[Compensation Panel]: Contains one negative (unstained) control tube and up to six positive (fluorochrome stained) control tubes. A compensation control tube is required for each fluorochrome used to mark samples in the sample group.
The icon indicates sample tubes for which data has been recorded or which have been used for sorting. These tubes cannot be reassigned as the active tube.
Double-clicking a tube displays the worksheet for the tube.

[Negative control]: Tube for recording data for the negative control sample used to compensate for cellular autofluorescence.

Positive control tubes: Tubes for recording single-stained positive control samples used to calculate the fluorescence compensation spillover matrix, one tube for each fluorochrome used to mark samples.

Sample tube: Tubes containing sample for data acquisition, recording, and cell sorting. Each tube also has worksheet settings and sort & stop settings associated with it.
Double-clicking a tube displays the worksheet for the tube.
To make a tube the active tube for data acquisition, select the tube and click [Assign Tube] in the [Target] group on the [Experiment] tab of the ribbon.
During acquisition the tube icon changes to .
The icon indicates sample tubes for which data has been recorded or which have been used for sorting. These tubes cannot be reassigned as the active tube.
The following icons are displayed for each tube on the right side in the Experiment Explorer.
: Indicates data is being recorded.
: Indicates data has been recorded.
: Indicates tube is being sorted.
: Indicates that the tube has been used for sorting into the collection tubes.
[Tube Information]: Contains information about the tube. Double-clicking this component displays the [Tube Information] dialog *(page 135)*.
[Worksheet Settings]: Contains information about the plots, gates, and statistics on the worksheet. Double-clicking this component displays the [Worksheet Settings] dialog *(page 135)*.
[Stop & Sort Settings]: Contains the sort settings, and the stop conditions for data acquisition, recording, and sorting for the tube. Double-clicking this component displays the [Stop & Sort Settings] dialog *(page 135)*.
Data source: Indicates the presence of saved data and type of sort results.

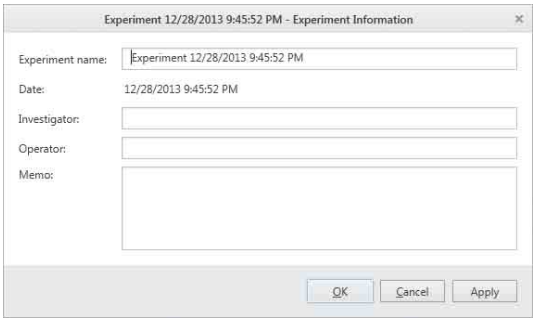
Data source naming convention

The sorting method is prefixed to the name of the data source.

Sorting method	Data source name
2 Way Tubes	[2 Way] Data Source - #
8 Well Strips	[8 Well] Data Source - #
Slides	[Slide] Data Source - #
6 Well Plate 12 Well Plate	[6 Well] Data Source - # [12 Well] Data Source - #
24 Well Plate	[24 Well] Data Source - #
48 Well Plate	[48 Well] Data Source - #
96 Well Plate	[96 Well] Data Source - #
384 Well Plate	[384 Well] Data Source - #
Index sorting Example: 96 Well Plate	[Index 96 Well] Data Source - # * The data source name has an "Index" prefix.
Imported data created using SH800 Software version 1.3.1 or earlierEx: 8 Well Strips	[Old 8 Well] Data Source - # * The data source name has an "Old" prefix.

[Experiment Information] dialog

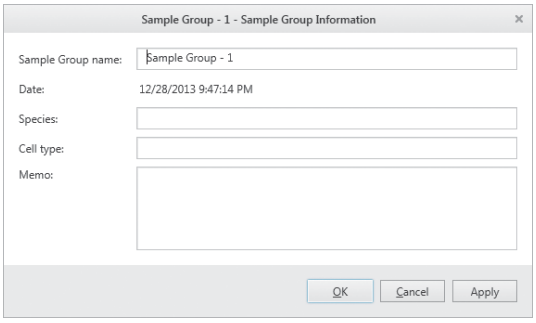
The [Experiment Information] dialog is displayed by double-clicking [Experiment Information] for an experiment in the Experiment Explorer.
The [Experiment Information] dialog is used to view and edit information about the experiment.



[Experiment Name]: Name of the experiment (required item).
[Date]: Displays the date the experiment was created. This parameter cannot be modified.
[Investigator]: Name of the lead researcher.
[Operator]: Name of the operator.
[Memo]: Descriptive comments.
[OK]: Applies the settings and closes the dialog.
[Cancel]: Cancels settings and closes the dialog.
[Apply]: Applies the settings without closing the dialog.

[Sample Group Information] dialog

The [Sample Group Information] dialog is displayed by double-clicking [Sample Group Information] in an experiment in the Experiment Explorer.
The [Sample Group Information] dialog is used to view and edit information about the sample group.

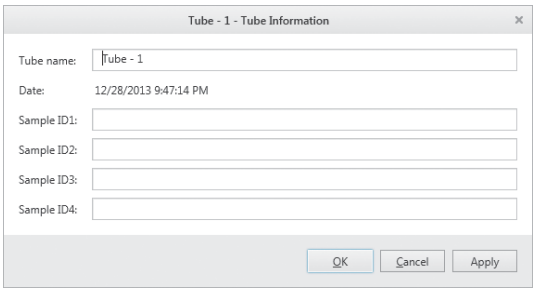


[Sample Group Name]: Unique name of the sample group (required item).

[Date]: Displays the date the sample group was created. This parameter cannot be modified.
[Species]: Name of the target biological species.
[Cell type]: Name of the cell type within the biological species.
[Memo]: Descriptive comments.
[OK]: Applies the settings and closes the dialog.
[Cancel]: Cancels settings and closes the dialog.
[Apply]: Applies the settings without closing the dialog.

[Tube Information] dialog

The [Tube Information] dialog is displayed by double-clicking [Tube Information] for a sample tube in the Experiment Explorer.
The [Tube Information] dialog is used to view and edit information about the sample tube.



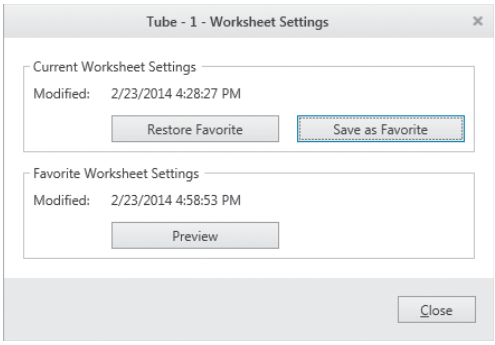
[Tube Name]: Unique name of the sample tube (required item).
[Date]: Displays the date the sample tube was created. This parameter cannot be modified.
[Sample ID1] to [Sample ID4]: ID labels for identifying the sample tube.
[OK]: Applies the settings and closes the dialog.
[Cancel]: Cancels settings and closes the dialog.
[Apply]: Applies the settings without closing the dialog.

[Worksheet Settings] dialog

The [Worksheet Settings] dialog is displayed by double-clicking [Worksheet Settings] for a sample tube in the Experiment Explorer.
The [Worksheet Settings] dialog is used to save the current worksheet settings (number of plots, type of plots, statistics settings, etc.) as the favorite settings. Saving current worksheet settings as the favorite settings allows you to restore the settings at a later time or to apply the settings to a different sample tube.

Tip

Data that is acquired and recorded for a tube is not included in the worksheet settings.



[Current Worksheet Settings]

[Modified]: Displays the date and time that the current worksheet settings were modified.
[Restore Favorite]: Restores the saved favorite worksheet settings as the current worksheet settings.
[Save as Favorite]: Saves the current worksheet settings as the favorite (default) worksheet settings.

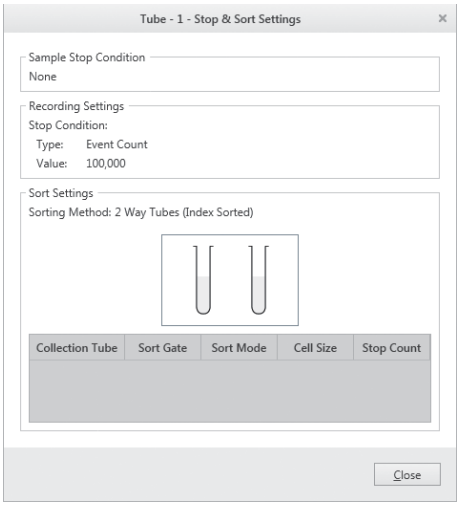
[Favorite Worksheet Settings]

Modified]: Displays the date and time that the favorite worksheet settings were saved.
[Preview]: Displays a preview of the worksheet with the saved favorite worksheet settings.
[Close]: Closes the dialog.

[Stop & Sort Settings] dialog

The [Stop & Sort Settings] dialog is displayed by double-clicking [Stop & Sort Settings] for a sample tube in the Experiment Explorer.
The stop and sort settings can be viewed in the [Stop & Sort Settings] dialog.

Example: 2-way tube sorting



[Sample Stop Condition]: Displays the data acquisition stop condition.

[Recording Settings]

[Stop Condition]: Displays the recording stop condition.

[Sort Settings]

[Sorting Method]: Displays the sorting method for the sample tube.
You can change the order items are displayed by dragging and dropping the title cells.

[Close]: Closes the dialog.

Sorting Control Pane

The sorting control pane is used to configure and control cell sorting.

For details, see "Sorting" (page 53).

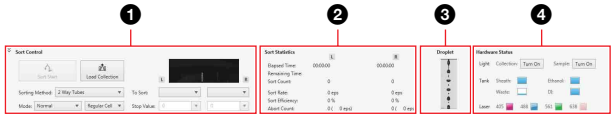
The items displayed in the sorting control pane vary depending on the selected sorting method. This section describes the displayed items using two-say tube sorting and 96-well plate sorting as examples.

Tip

You can show/hide the sorting control pane by clicking in the top left corner of the pane. In analyzer mode, the sorting control pane is automatically hidden.

For 2-way tube sorting

The following items are displayed when [2 Way Tubes] is selected in [Sorting Method].



❶ [Sort Control] pane

Configures and controls droplet sorting.

[Sort Start] / [Sort Stop]: Starts/stops sorting.

[Load Collection] / [Unload Collection]: Loads/unloads the collection stage.

[Sorting Method]: Selects the sorting method. Set the sorting method to match the collection devices loaded on the collection stage.
The sorting parameters vary depending on the selected sorting method.

[Mode]: Selects the sorting mode.

[Cell Size]: Displays the current cell size setting.

Sorting mode

- Ultra Purity
- Purity
- Semi-Purity
- Normal
- Semi-Yield
- Yield
- Ultra Yield
- Single Cell

Cell size

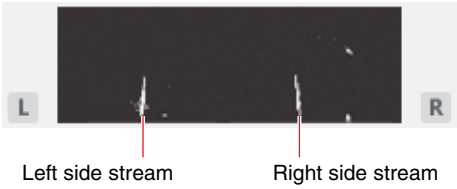
- Regular Cell
- Large Cell

For details about each sorting mode, see "Sorting Mode" (page 93).

Tips

- When a "Large cell" option is selected, the side stream deflection angle is adjusted for sorting of cells of larger size.
- The "Large cell" options are not displayed if sorting calibration is not performed. Select a "Large cell" option if droplet formation is irregular, or the droplet stream is hitting the side wall of the collection tubes or is missing the tubes when sorting cells larger than 15 µm in Normal mode.
- The side streams may become unstable when sorting cells of size 20 µm or larger.
- If droplet marks are visible on the collection tube holder after sorting, wipe clean with a lint-free, soft, clean cloth sprayed with ethanol and then another moistened with water.

Side stream monitor: Displays the image from the side stream monitor camera.



For details about adjusting the deflection angle of side streams, see "[Advanced Settings] dialog" (page 122).

[To Sort]: Selects the gates for the target events to sort into the left and right collection tubes.

[Stop Value]: Specifies the event count conditions to stop recording automatically for each of the left and right collection tubes.
You can select a value or enter a value from the keyboard.
Entering a value of "0" disables the stop condition.

❷ [Sort Statistics] pane

Displays the statistics about events during sorting.

[Elapsed Time]: Displays the cumulative elapsed time since the start of sorting.

[Remaining Time]: Displays the estimated remaining sorting time.

[Sort Count]: Displays the number of events sorted.

[Sort Rate]: Displays the rate at which events are detected during sorting in units of events per second (eps).

[Sort Efficiency]: Displays the number of sorts attempted as a percentage of targeted cells in the sample.

[Abort Count]: Displays the number of events that do not match the sort criteria and which are aborted during sorting.

[Abort Rate]: Displays the rate at which events are aborted during sorting in units of events per second (eps).

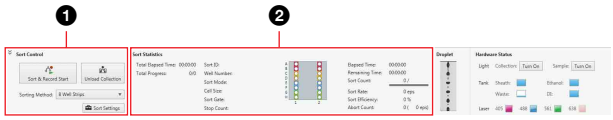
❸ [Droplet] pane
Displays the droplet stream immediately beneath the sorting chip nozzle.
The droplet stream is used to monitor droplet formation and to adjust the breakoff point.
Double-clicking the image displays the side streams in the [Droplet Viewer] window *(page 138)* for monitoring sorting.

❹ [Hardware Status] pane
Displays the status of the interior lights in the sample loader and collection stage area, and the level in each of the tanks in the fluidics cart. It also displays the lasers enabled for the experiment.

[Light]
[Collection]: Turns the collection area light on/off.
[Sample]: Turns the sample chamber light on/off.
Tank: Indicates the fluid level in the sheath tank, ethanol tank, waste tank, and DI water tank.
A warning icon appears when the waste tank is close to full, or when the sheath, ethanol, or DI water tank is close to empty.
[Laser]: Indicates the lasers used for data acquisition.

For 8-well strips or slide glass sorting

The following items are displayed when [8 Well Strips] or [Slides] is selected in [Sorting Method].



The [Droplet] and [Hardware Status] panes are the same as for two-way tube sorting.

❶ [Sort Control] pane
Configures and controls droplet sorting.
[Sort Start] / [Sort Stop]: Starts/stops sorting.
[Load Collection] / [Unload Collection]: Loads/unloads the collection stage.
[Sorting Method]: Selects the sorting method. Set the sorting method to match the collection devices loaded on the collection stage.
The sorting parameters vary depending on the selected sorting method.
[Sort Settings]: Specifies the default sorting configuration.
Clicking this button displays the [Sort Settings] dialog.

For details about the [Sort Settings] dialog, see "Sorting" (page 53) and "Adjusting the Sort Position (SH800ZP only)" (page 99).

❷ [Sort Statistics] pane
Displays the statistics about events during sorting.
[Total Elapsed Time]: Displays the cumulative elapsed time since the start of sorting of the whole plate.
[Total Progress]: Displays the ratio of sorted wells to the total number of wells.
[Sort ID]: Displays the ID of the well currently being sorted.
[Well Number]: Displays the number of the well currently being sorted.
[Sort Mode]: Displays the sorting mode of the well currently being sorted.
[Cell Size]: Displays the current cell size setting.
[Sort Gate]: Displays the sort gate of the well currently being sorted.
[Stop Count]: Displays the number of events for the automatic stop condition of the well currently being sorted.
Plate (image): Displays the layout of the wells.
Double-clicking the plate image or right-clicking and selecting [Show Sorting Report] from the context menu displays the plate image in a separate window for monitoring progress.
[Elapsed Time]: Displays the cumulative elapsed time since the start of sorting.
[Remaining Time]: Displays the estimated remaining sorting time.
[Sort Count]: Displays the number of events sorted.
[Sort Rate]: Displays the rate at which events are detected during sorting in units of events per second (eps).
[Sort Efficiency]: Displays the number of sorts attempted as a percentage of targeted cells in the sample.
[Abort Count]: Displays the number of events that do not match the sort criteria and which are aborted during sorting.
[Abort Rate]: Displays the rate at which events are aborted during sorting in units of events per second (eps).

For multi-well plate sorting

The following items are displayed when a multi-well plate is selected in [Sorting Method].

Example: When [96 Well Plate] is selected

The [Droplet] and [Hardware Status] panes are the same as for two-way tube sorting.

❶ [Sort Control] pane
Configures and controls droplet sorting.
[Sort Start] / [Sort Stop]: Starts/stops sorting.
[Load Collection] / [Unload Collection]: Loads/unloads the collection stage.
[Sorting Method]: Selects the sorting method. Set the sorting method to match the collection devices loaded on the collection stage.
The sorting parameters vary depending on the selected sorting method.

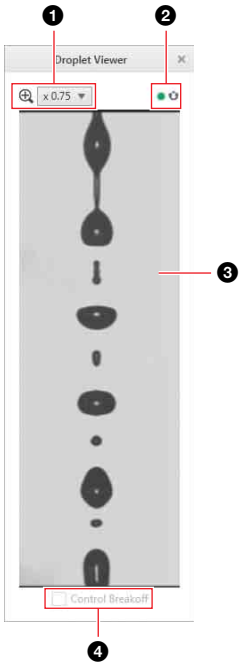
[Sort Settings]: Specifies the default sorting configuration.
Clicking this button displays the [Sort Settings] dialog.

For details about the [Sort Settings] dialog, see "Sorting" (page 53) and "Adjusting the Sort Position (SH800ZP only)" (page 99).

❷ [Sort Statistics] pane
Displays the statistics about events during sorting.
[Total Elapsed Time]: Displays the cumulative elapsed time since the start of sorting of the whole plate.
[Total Progress]: Displays the ratio of sorted wells to the total number of wells.
[Sort ID]: Displays the ID of the well currently being sorted.
[Well Number]: Displays the number of the well currently being sorted.
[Sort Mode]: Displays the sorting mode of the well currently being sorted.
[Cell Size]: Displays the current cell size setting.
[Sort Gate]: Displays the sort gate of the well currently being sorted.
[Stop Count]: Displays the number of events for the automatic stop condition of the well currently being sorted.
Plate (image): Displays the layout of the wells.
Double-clicking the plate image or right-clicking and selecting [Show Sorting Report] from the context menu displays the plate image in a separate window for monitoring progress.
[Elapsed Time]: Displays the cumulative elapsed time since the start of sorting.
[Remaining Time]: Displays the estimated remaining sorting time.
[Sort Count]: Displays the number of events sorted.
[Sort Rate]: Displays the rate at which events are detected during sorting in units of events per second (eps).
[Sort Efficiency]: Displays the number of sorts attempted as a percentage of targeted cells in the sample.
[Abort Count]: Displays the number of events that do not match the sort criteria and which are aborted during sorting.
[Abort Rate]: Displays the rate at which events are aborted during sorting in units of events per second (eps).

[Droplet Viewer] window

The [Droplet Viewer] window is displayed by double-clicking the image in the [Droplet] pane at the bottom of the main window.
The [Droplet Viewer] window is used to monitor droplet formation and as a guide when adjusting the breakoff point.



❶ Zoom factor
Selects the zoom factor (×1.00, ×0.75, or ×0.50)

❷ Status icon
: Indicates the stability of droplet formation.

Indication	Status
Flashing slowly	Waiting for stream to stabilize
Flashing rapidly	Adjustment in progress
On	Stable

: Indicates that the droplet control breakoff function is active.
No icon: Indicates unstable or absence of droplet formation.

❸ Droplet monitor
Displays the droplet stream.

❹ [Control Breakoff]
The control breakoff setting uses a feedback system to control the breakoff position automatically.
When enabled, the amplitude and frequency settings are adjusted automatically to maintain a stable breakoff point position with coherent side streams. When adjusting the breakoff point, first set the amplitude and frequency settings to obtain a stable breakoff point. After establishing a stable breakoff point, click the feedback control checkbox to enable the breakoff feedback control.

Tips

- The manual amplitude and frequency controls are disabled in this mode.
- When breakoff feedback control is enabled, if the breakoff point moves and cannot be recovered within a predetermined time, sorting stops and an error message is displayed on the screen.

Context Menu

The context menu displays common operations that can be accessed by right-clicking components in the Experiment Explorer or items on the worksheet.

Experiment Explorer Context Menu

This section describes the context menu items displayed for components in the Experiment Explorer.

Experiments

The following menu commands are displayed when right-clicking an experiment icon in the Experiment Explorer.

[New Sample Group]: Creates a blank sample group.
[Copy] / [Paste]: Copies the experiment and pastes a new experiment into the Experiment Explorer.
[Duplicate]: Duplicates the selected experiment.
[Save as Template]: Saves the selected experiment as a private template.
[Export FCS File]: Displays the [FCS File Export] dialog *(page 117)* for exporting the tubes in the selected experiment in FCS 3.0 or FCS 3.1 format.
[Send to Public]: Creates a duplicate of the selected experiment and places the duplicate under the [Public] node for sharing with other users.
[Delete]: Deletes the selected experiment.
[Rename]: Renames the selected experiment.

Compensation Panel

The following menu commands are displayed when right-clicking a compensation panel icon in the Experiment Explorer.

[Compensation Wizard]: Displays the [Compensation Wizard] *(page 61)* for recording control tubes in a compensation panel.
[Show Matrix]: Displays the [Compensation Settings] dialog *(page 124)* for viewing the spillover matrix, and loading/saving the spillover matrix.
[Calculate Matrix] Displays the [Calculate Compensation Settings] dialog *(page 125)* for calculating the coefficients of the fluorescence compensation spillover matrix based on the negative control tubes and the compensation panel.
[Export FCS File]: Displays the [FCS File Export] dialog *(page 117)* for exporting the tubes in the selected compensation panel in FCS 3.0 or FCS 3.1 format.

Control tubes in a compensation panel

The following menu commands are displayed when right-clicking a control tube in a compensation panel icon in the Experiment Explorer.

[Assign]: Assigns the selected control tube as the active tube for data acquisition and recording.
[Open Worksheet]: Opens the worksheet tab for the selected control tube.
[Export FCS File]: Displays the [FCS File Export] dialog *(page 117)* for exporting the selected control tube in FCS 3.0 or FCS 3.1 format.
[Reset Data]: Deletes the data recorded for the control tube.

Sample groups

The following menu commands are displayed when right-clicking a sample group icon in the Experiment Explorer.

[New Tube]: Creates a sample group.
Selecting this item creates a new sample group for the experiment.
[Copy] / [Paste]: Allows you to copy and paste all settings (including sample tubes) in the selected sample group. However, the recorded data of the sample tubes will not be pasted.
[Paste to All Tubes in Sample Group]: Pastes copied worksheet settings to all sample tubes in the selected sample group.
This item is available when you copy the worksheet settings of a sample group, and then right-click another sample group.
[Duplicate]: Duplicates the selected sample group.
[Save as Template]: Displays the [Save as Template] dialog *(page 115)* for saving the selected sample group as a private template.
[Apply Template]: Displays the [Apply Template] dialog *(page 115)* for selecting a template to apply to the selected sample group.
[Export FCS file]: Displays the [FCS File Export] *(page 117)* dialog for exporting the recorded data for the sample tubes included in the sample group as an FCS 3.0 or FCS 3.1 format file.
[Delete]: Deletes the selected sample group.
[Rename]: Renames the selected sample group.

Measurement settings

The following menu commands are displayed when right-clicking a [Measurement Settings] icon for a sample group in the Experiment Explorer.

[Copy] / [Paste]: Copies the measurement settings for the selected sample group and pastes them into the measurement settings for another sample group.
[Show Settings]: Displays the [Measurement Settings] dialog *(page 73)* for viewing measurement settings.

Compensation settings

The following menu commands are displayed when right-clicking a [Compensation Settings] icon for a sample group in the Experiment Explorer.

[Copy] / [Paste]: Copies the compensation settings for the selected sample group and pastes them into the compensation settings for another sample group.
[Show Settings]: Displays the [Compensation Settings] dialog *(page 124)* for viewing compensation settings.

Sample tubes

The following menu commands are displayed when right-clicking a sample tube icon in the Experiment Explorer.

[Assign]: Assigns the selected sample tube as the active tube for data acquisition, recording, and cell sorting.
[Open Worksheet]: Opens the worksheet tab for the selected sample tube.
[Apply Compensation]: Applies fluorescence compensation to the plots on the worksheet for the selected sample tube.
[Copy] / [Paste]: Copies the sample tube and pastes it into another sample tube.
[Duplicate]: Duplicates the selected sample tube.
[Save as Template]: Saves the selected sample tube as a private template.
[Apply Template]: Displays the [Apply Template] dialog *(page 115)* for selecting a template to apply to the selected sample tube.
[Export FCS File]: Displays the [FCS File Export] dialog *(page 117)* for exporting the selected sample tube in FCS 3.0 or FCS 3.1 format.
[Delete]: Deletes the selected sample tube.
[Rename]: Renames the selected sample tube.
[Show Plate Sorting Monitor]: Displays the [Plate Sorting Monitor] dialog *(page 60)* for monitoring the status when sorting onto multi-well plates.
[Show Results]: Displays the [Tube Results] dialog *(page 115)* for displaying the recording results and sorting results for the selected sample tube.

Tube worksheet settings

The following menu commands are displayed when right-clicking a [Worksheet Settings] icon for a sample tube in the Experiment Explorer.

[Copy] / [Paste]: Copies the worksheet sheet settings for the selected sample tube and pastes them into the worksheet settings for another sample tube.
[Save as Favorite]: Saves the current worksheet settings as the favorite (default) worksheet settings.
[Restore Favorite]: Restores the saved favorite worksheet settings as the current worksheet settings.
[Apply to All Tubes In Same Sample Group]: Applies the worksheet settings for the selected sample tube to all sample tubes within the sample group.
[Show Settings]: Displays the [Worksheet Settings] dialog *(page 135)* for displaying, saving, and loading worksheet settings.

Data sources

The following menu commands are displayed when right-clicking a data source icon in the Experiment Explorer.

[Open Data Source]: Displays the data source on the worksheet.
[Delete]: Deletes the selected data source.
[Rename]: Renames the selected sample tube.
[Export FCS File]: Displays the [FCS File Export] dialog *(page 117)* for exporting the selected data source in FCS 3.0 or FCS 3.1 format.
[Show Results]: Displays the [Data Source Results] dialog *(page 115)* for displaying the recording results and sorting results for the selected data source.

Worksheet Context Menu

This section describes the context menu items displayed for items displayed on the worksheet and the worksheet itself.

Worksheet

The following menu commands are displayed when right-clicking on the worksheet.

[Refresh]: Updates the information displayed on the worksheet.
[New Density]: Adds a new density plot to the worksheet.
[New Dot Plot]: Adds a new dot plot to the worksheet.
[New Histogram]: Adds a new histogram plot to the worksheet.
[Show Table]: Shows/hides the [Gates and Statistics] table.
[Show Grid]: Displays the grid on the worksheet.
[Snap to Grid]: Aligns the plots and [Gates and Statistics] table on the worksheet with the grid when they are moved or added.
[Auto Arrange]: Automatically aligns the plots and [Gates and Statistics] table on the worksheet to the grid.
[Fit to Screen]: Expands the worksheet to fill the entire screen.
[Edit Gate]: Displays the [Gate Editor] dialog *(page 127)* to edit gates.
[Edit Statistics]: Displays the [Statistics Editor] dialog *(page 92)* to configure the statistics displayed in the [Gates and Statistics] table.

[Favorite Settings] >

[Save as Favorite]: Saves the current worksheet settings as the favorite (default) worksheet settings.

[Restore Favorite]: Restores the saved favorite worksheet settings as the current worksheet settings.

[Copy Worksheet Picture]: Copies the selected plot or [Gates and Statistics] table to the clipboard for pasting in other software, for example to produce reports.

[Print]: Displays the [Print] window *(page 108)* for printing the analysis results displayed on the worksheet.

[Custom Print]: Displays the [Custom Print] dialog *(page 128)* for selecting the items to print.

[Properties]: Displays the [Property Window] dialog *(page 130)*.

Plots

The following menu commands are displayed when right-clicking a plot.

[Refresh]: Updates the information displayed on the worksheet.

[Auto Adjust]: Automatically adjusts the ranges of the X axis and Y axis of all plots to their minimum and maximum measured values.

[Remove]: Removes the selected plot.

[Duplicate]: Duplicates the selected plot.

[Copy All Regions] / [Paste Region]: Copies all gates on a plot and pastes them onto another plot.

[Create Gate] >

[Rectangle]: Creates a rectangular gate on a dot plot or density plot.

[Ellipse]: Creates an elliptical gate on a dot plot or density plot.

[Polygon]: Creates a polygonal gate on a dot plot or density plot.

[Quadrant]: Creates a quadrant gate on a dot plot or density plot.

[Linear]: Creates a linear gate on a histogram plot.

[Plot Type] >

[Density]: Changes the type of the selected plot to a density plot.

[Dot Plot]: Changes the type of the selected plot to a dot plot.

[Histogram]: Changes the type of the selected plot to a histogram plot.

[Zoom]: Zooms in on the area selected by dragging the mouse on a plot.

[Open Gates Editor]: Displays the [Gate Editor] dialog *(page 127)* to edit gates.

[Open Statistics Editor]: Displays the [Statistics Editor] dialog *(page 92)* to configure the statistics displayed in the [Gates and Statistics] table.

[Copy Picture]: Copies the selected plot to the clipboard for pasting in other software, for example to produce reports.

[Properties]: Displays the [Property Window] dialog *(page 130)*.

Gates

The following menu commands are displayed when right-clicking a gate.

[Create Density Plot]: Adds a density plot for the events within the selected gate.

[Create Dot Plot]: Adds a dot plot for the events within the selected gate.

[Create Histogram Plot]: Adds a histogram plot for the events within the selected gate.

[Move Color Order to Front]: Moves the selected gate to the front.

[Move Color Order to Back]: Moves the selected gate to the back.

[Convert to] >

[Rectangle]: Converts the selected gate to a rectangle gate.

[Ellipse]: Converts the selected gate to an ellipse gate.

[Polygon]: Converts the selected gate to a polygon gate.

[Copy Region] / [Paste Region]: Copies the selected gate and pastes it into another plot.

[Visible Gate]: Shows/hides the selected gate.

[Remove]: Deletes the selected plot.

[Send to Back]: Moves the selected gate to the back.

[Properties]: Displays the [Property Window] dialog *(page 130)*.

[Gates and Statistics] table

The following menu commands are displayed when right-clicking within the [Gates and Statistics] table.

[Show Table]: Shows/hides the [Gates and Statistics] table.

[Save as CSV File]: Displays the [Exporting statistics table] dialog *(page 128)* for exporting the data in the [Gates and Statistics] table as a CSV-format file.

[Copy Picture]: Copies the [Gates and Statistics] table to the clipboard for pasting in other software, for example to produce reports.

[Open Gates Editor]: Displays the [Gate Editor] dialog *(page 127)* to edit gates.

[Open Statistics Editor]: Displays the [Statistics Editor] dialog *(page 92)* to configure the statistics displayed in the [Gates and Statistics] table.

[Gates and Statistics] table cells

The following menu commands are displayed when right-clicking within a [Gates and Statistics] table cell.

[Create Density Plot]: Adds a density plot for the events within the selected gate.

[Create Dot Plot]: Adds a dot plot for the events within the selected gate.

[Create Histogram Plot]: Adds a histogram plot for the events within the selected gate.

[Create Parent Plot]: Adds a parent plot to the worksheet.

[Copy Selected Gate]: Copies the selected gate.

[Cut Selected Gate]: Cuts the selected gate.
[Paste Selected Gate]: Pastes a previously copied or cut gate.
[Visible Gate]: Shows/hides the selected gate.
[Remove Selected Gate]: Deletes the selected gate.
[Show Table]: Shows/hides the [Gates and Statistics] table.
[Save as CSV File]: Displays the [Exporting statistics table] dialog *(page 128)* for exporting the data in the [Gates and Statistics] table as a CSV-format file.
[Open Gate Editor]: Displays the [Gate Editor] dialog *(page 127)* to edit gates.
[Open Statistics Editor]: Displays the [Statistics Editor] dialog *(page 92)* to configure the statistics displayed in the [Gates and Statistics] table.
[Refresh List]: Updates the content displayed in the [Gates and Statistics] table.
[Properties]: Displays the [Property Window] dialog *(page 130)*.

Maintenance

Chapter 8

This chapter describes the routine maintenance procedures required to maintain accurate performance and longevity of the SH800.

DANGER

Fluids may contain biological, chemical, or other agents. Always wear gloves and other protective clothing, mask, and goggles, as required, when performing maintenance.

Maintenance Schedule

The maintenance procedures outlined in this manual should be performed according to the schedule below.

Preparations for the Day Before Measurement

Maintenance item	Schedule	See
Refilling the sheath tank	• It is recommended to fill the tank the day before operation to allow air bubbles to escape.	*"Refilling the Sheath Tank" (page 146)*

Maintenance for Each Startup Operation

Maintenance item	Schedule	See
Disposing of waste fluid Replacing the waste tank	• Check that the tank is not full when starting up and shutting down • Replace when nearly full or when prompted in SH800 software	*"Emptying/ Changing the Waste Tank" (page 148)*
Checking that there is sufficient volume in the sheath tank	• When starting up	*"Refilling the Sheath Tank" (page 146)*
Refilling the sheath tank	• When prompted in SH800 Software.	
Refilling the DI water tank	• When starting up • When prompted in SH800 Software • As required	*"Refilling the DI Water Tank" (page 149)*

Maintenance for Each Shutdown Operation

Sample fluidics system cleaning

Maintenance item	Schedule	See
Sample fluidics system cleaning	• When shutting down at day's end • As required	*"Cleaning the Sample Fluidics System using Bleach" (page 152)*

Tip

Use the same procedure above to clean the sample line and sample probe, even on models that do not support the PEEK sample line.

Parts cleaning

Maintenance item	Schedule	See
Cleaning the sample loader	• When shutting down	*"General Cleaning" (page 153)*
Cleaning the sorting area and transparent protective covers	• When shutting down	
Cleaning the droplet camera windows	• When shutting down	
Cleaning the laser windows	• When shutting down	
Cleaning the collection tube holders, multi-well plate holder	• When shutting down • As required	
Cleaning the splash guard	• When shutting down • As required	

Monthly Maintenance

Maintenance item	Schedule	See
Changing the waste tank air filter	• Monthly	*"Changing Tank Air Filters" (page 165)*
Cleaning the DI water tank and DI water line	• Monthly • As required	*"Cleaning the DI Water Tank and DI Water Line" (page 166)*
Running ethanol cleaning	• Monthly • As required	*"Running ethanol cleaning" (page 158)*

Periodic Maintenance

Maintenance item	Schedule	See
Changing the PEEK sample line or the sample line	• Every 3 months • As required	*"Changing the PEEK Sample Line (PEEK sample line compatible models)" (page 169)* *"Changing the Sample Line and Probe (Non-PEEK sample line compatible models)" (page 172)*
Autoclaving the probe adapter and sample probe	• Every 3 months • As required	
Changing the sheath filter	• Every 3 to 12 months • As required	*"Changing the Sheath Filter" (page 176)*
Changing the DI water filter	• Every 3 to 12 months • As required	*"Changing the DI Water Filter" (page 177)*
Changing the sample loader O-ring	• Every 3 to 6 months	*"Changing the Sample Loader O-Ring" (page 160)*
Changing the sample probe	• Every 12 months	*"Changing the Sample Line and Probe (Non-PEEK sample line compatible models)" (page 172)*
Changing the probe adapter	• Every 12 months	*"Changing the Probe Adapter (PEEK sample line compatible models)" (page 175)*
Changing the sintered sheath line filter	• Every 12 months	*"Changing the Sintered Sheath Line Filter" (page 179)*
Changing the sheath tank air filter	• Every 12 months	*"Changing Tank Air Filters" (page 165)*
Changing the DI water tank and ethanol tank air filter	• Every 12 months	
Changing the waste catcher O-ring	• Every 12 months	*"Cleaning the Waste Catcher" (page 157)*

Miscellaneous

Maintenance item	Schedule	See
Cleaning the deflection plates	• As required (when dirty)	*"Cleaning the Deflection Plates" (page 155)*
Cleaning the waste catcher	• As required (when dirty)	*"Cleaning the Waste Catcher" (page 157)*
Running Waste Line A maintenance	• After a week or more of non-use· • As required	*"Running Waste Line A Maintenance" (page 159)*
Cleaning the sample loader O-ring	• As required	*"Changing the Sample Loader O-Ring" (page 160)*
Cleaning the sheath tank and sheath line	• As required	*"Cleaning the Sheath Tank and Sheath Line" (page 162)*
Releasing air trapped in the sheath filter and sorting chip	• As required	*"Releasing Air in the Sheath Filter and Sorting Chip" (page 181)*
Releasing air trapped in the DI water filter	• As required	*"Releasing Air in the DI Water Filter" (page 182)*
Autoclaving the sheath filter and DI water filter	• As required	*"Autoclaving the Sheath Filter and DI Water Filter" (page 183)*
Ejecting the sorting chip manually	• As required	*"Ejecting the Sorting Chip Manually" (page 185)*
Cleaning the optical filters	• As required	*"Cleaning and Handling of Optical Filters" (page 186)*
Disconnecting and reconnecting the fluidics cart	• As required	*"Disconnecting and Reconnecting the Fluidics Cart" (page 187)*
Cleaning the internal sheath and DI water line with ethanol	• As required	*"Cleaning the Internal Sheath Line and DI Water Line using Ethanol" (page 188)*

Fluidics Cart

Status of fluidics cart tank

The status of the three fluidics tanks in the fluidics cart is displayed on the LCD monitor, in addition to the Hardware Status pane in the bottom right corner of the main window in SH800 Software.

Fluidics cart lock mechanism

The fluidics cart is fitted with a simple lock to prevent the tray from being withdrawn during operation. The cart should only be unlocked when performing fluidics cart maintenance. The cart can be locked/unlocked using a coin, or similar flat object.

Refilling the Sheath Tank

The sheath tank should be checked every time before sorting and refilled if it is close to empty. Also, the tank must be refilled when prompted to do so in SH800 Software.

Notes

- Sheath fluid is a sterile solution. Avoid contact with the contents of the tank to maintain sheath fluid sterility.
- Never use the supplied sheath tank for any other purpose.
- When opening the sheath tank cap, check that "Standby" is displayed on the LCD monitor or that the power to the main unit is turned off. If "Standby" is not displayed, place the unit in Standby mode using the following procedure.
  ① Click [Settings] on the [Cytometer] tab of the ribbon.
  The [Cytometer Settings] dialog appears.
  ② Click [Advanced Settings].
  The [Advanced Settings] dialog appears.
  ③ Click [Standby] on the [Pressure Options] tab.
  ④ Check that "Standby" is displayed on the LCD monitor.
- Do not force the lid down or place objects on the lid when the tank is pressurized.
- Store replacement sheath fluid in a room with the same temperature environment as the instrument, or allow the sheath fluid to acclimatize in the same room as the instrument before refilling the sheath tank. Droplet formation may become unstable if the sheath fluid added to the tank is a different temperature to the sheath fluid remaining in the tank.

Note

It is recommended that the sheath tank be filled one day before measurement to ensure all air bubbles are removed from the sheath fluid.

1 Pull out the fluidics cart tray.

2 Disconnect the sheath air line (clear) from the top of the sheath tank.

Note

It is important to disconnect the sheath air line before disconnecting the sheath fluid line.

3 Release the residual air pressure from the tank using the ring-pull air relief valve, then remove the tank from the tray.

4 Disconnect the sheath fluid line (blue) from the top of the tank.

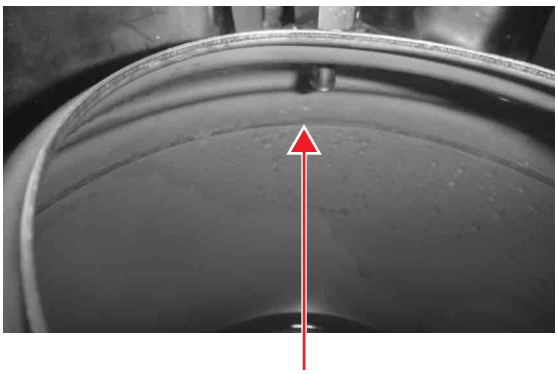
5 Lift the lever securing the lid of the tank to open the lid.

6 Lower the lid slightly into the tank, turn it 90 degrees, and then remove the lid from the tank.

7 Refill the tank with sheath fluid.

Notes

- Do not touch the surfaces of the sheath tank lid that come into contact with sheath fluid. Take care not to spill any fluid.
- Do not use a pump or other object that has not been sterilized for filling the tank. It is recommended that the tank be placed immediately beneath the tap of the source sheath fluid container so that the tank is filled using gravity alone.
- Overfilling the sheath tank may damage the unit. Do not fill the tank beyond the full line inside the tank.



Fill with sheath fluid to below this line.

8 Insert and secure the lid, place the tank in the cart tray, and connect the sheath air line (clear) and sheath fluid line (blue) in the reverse order of removal, making sure the connectors are inserted until they lock into place.

Note

When inserting the sheath tank lid, make sure that the black rubber seal is attached to the lid.

9 Carefully clean any residue fluid from the connectors and outside surfaces of the tank and stow the tray.

Note

Always place the tank in the center of the tray area. The fluid level detector may not operate correctly if the tank is placed off-center.

10 If the sheath tank was refilled while the instrument is in use, click [Settings] on the [Cytometer] tab of the ribbon to display the [Advanced Settings] dialog, and then click [Ready] on the [Pressure Options] tab.

Note

To continue sorting, click [Sort Calibration] on the [Cytometer] tab of the ribbon to run auto calibration.

For details, see "Automatic Calibration" (page 46).

Emptying/Changing the Waste Tank

Check the fluid level in the waste tank daily before starting. If it is full, dispose of the waste fluid or replace the tank with a new tank. Also, the waste fluid must be disposed or the waste tank must be replaced when prompted to do so in SH800 Software.

Note

When handling the waste tank, always follow the laboratory rules for the handling of waste fluids.

DANGER

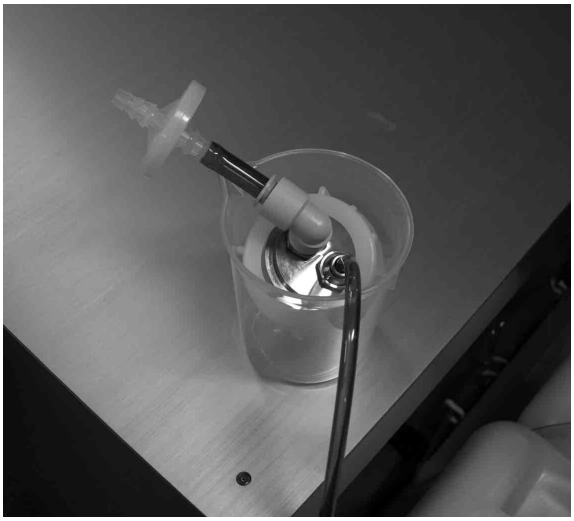
Waste fluid may contain virulent or hazardous substances that may be highly contagious or toxic, depending on the sample under test. Always wear gloves and other protective clothing, mask, and goggles, as required, when handling waste fluid.

1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel of the main unit.

2 Pull out the fluidics cart tray.

3 Unscrew the cap on the top of the waste tank and remove the waste tank nozzle.

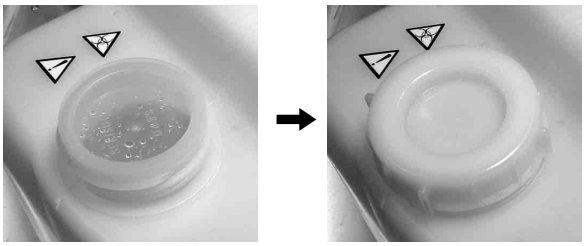
4 Place the cap (with nozzle, connector, and line attached) in a beaker or other container to prevent waste fluid spills from the nozzle.



Notes

- Do not touch the surfaces of the waste tank cap and tank nozzle that come into contact with waste fluid. Take care not to spill any fluid.
- Place the beaker in a safe position where it cannot be accidentally moved or tipped over.

5 Attach the inner cap on the tank, taking care not spill any waste fluid, then attach the outer cap and securely tighten.



The inner and outer stopper caps are supplied with the tank. Always attach both the inner and outer caps to ensure the tank is sealed.

6 Remove the waste tank from the fluidics cart tray.

7 Empty the tank or prepare a new waste tank.

Note

Dispose of the waste tank and fluid in accordance with the laboratory rules and local ordinances and regulations.

8 Place an empty waste tank or a new tank on the fluidics cart tray.

9 Insert the waste tank nozzle from the beaker into the tank, securely tighten the cap, and stow the tray.

Notes

- Make sure that the waste fluid line is connected securely to the connector on the cap of the waste tank, and that the cap is securely fastened on the waste tank to prevent leakages.
- Always place the tank in the center of the tray area. The fluid level detector may not operate correctly if the tank is placed off-center.
- Do not autoclave fluid tanks. The shape of the fluid tanks will deform if placed in an autoclave.
- If the air filter attached to the waste tank becomes blocked, air cannot escape the waste tank as it fills with waste fluid, which may cause incorrect operation. The air filter may become blocked if it gets wet. Do not tilt or otherwise jostle the waste tank if it is close to full.

Refilling the DI Water Tank

The DI water tank should be refilled daily before starting the unit. The tank should also be refilled as required if the level becomes too low during operation and before cleaning with ethanol.

Tip

The DI water tank can be refilled at any time, except during auto calibration, measurement, and cleaning.

Precautions when handling DI water

- Refill the DI water tank with fresh DI water every time when starting up, replacing any unused DI water in the tank.
- Regularly clean the DI water tank with ethanol to suppress the growth of bacteria *(page 166)*.
- Use ultra pure water or distilled water with extremely little or no bacteria or biological content to further suppress the growth of bacteria.

1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel.

2 Open the fluidics maintenance door, then disconnect the line from the top of the DI water tank.

Press the metal release catch to withdraw the connector.

3 Undo the metal restraining clamp and remove the DI water tank.

Note

Always wear gloves and other protective clothing, mask, and goggles, when handling fluidics system components. Skin cells and hair are the most common cause of bacterial contaminations.

4 Remove the cap, and withdraw the tank probe.

5 Discard any remaining DI water.

Note

Dispose of DI water in accordance with laboratory rules, and with local and federal ordinances and regulations.

6 Refill the tank with fresh DI water up to the opening of the tank, and reattach the cap.

7 Re-install the DI water tank.

Install in the reverse order of removal.

8 Check the drip tray for any fluid spills or leakages, and clean as necessary.

9 Close the door, and turn on the power supply.

Note

The DI water tank is fitted with an air filter that should be replaced every 12 months. For details, see *"Changing Tank Air Filters" (page 165)*.

Refilling the Ethanol Tank

The level of fluid in the ethanol tank can be monitored in the main window in SH800 Software and on the LCD monitor.

Precautions when handling ethanol

- Ensure the laboratory is well ventilated during cleaning with ethanol. Inhaling ethanol vapor can cause irritation of the eyes, skin and respiratory tract, loss of coordination, drowsiness, and in sufficient concentration, unconsciousness.
- Never place a naked flame, embers, or other material that can emit sparks near the main unit or the fluidics cart. Ethanol vapor is highly flammable at normal room temperature.
- Never pour ethanol into a tank other than the ethanol tank, and conversely, never pour other liquids into the ethanol tank.
- Make sure that the cap on the ethanol tank is correctly and securely fastened at all times.
- Make sure that the ethanol line is securely connected to the connectors on the rear panel of the main unit, rear panel of the fluidics cart, and the ethanol tank itself.
- When the ethanol tank is not in use, remove all ethanol from the tank.

Note

The SH800 is compatible with standard disinfectant ethanol (70%) intended for use in flow cytometers.

1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel of the main unit.

2 Pull out the fluidics cart tray.

3 Disconnect the ethanol line from the cap on the ethanol tank, and place it in a clean beaker or other container.

Press the metal release catch to withdraw the connector.

Note

Do not touch the surfaces of the ethanol connector that come into contact with ethanol. Take care not to spill any fluid.

4 Remove the ethanol tank from the fluidics cart for easy access to the cap of the tank.

5 Remove the cap and tank probe from the ethanol tank and place in a clean, safe location.

6 Refill the ethanol tank with the specified volume of ethanol, and reattach the cap.

7 Place the tank in the fluidics cart tray, connect the ethanol line, and stow the tray.

Insert the line connector and push until it locks into place. Carefully clean any residue fluid from the connector and outside surfaces of the tank.

Cleaning the Sample Fluidics System using Bleach

The sample line and probe should be cleaned regularly using a protein-dissolving bleach to disinfect the lines that carry the sample fluid. The fluidics system should be disinfected on a regular basis, or as required, especially if running samples that contain, for example, infectious substances.

The sorting chip, PEEK sample line, sample line, and sample probe can be disinfected using a 1 to 3% Sodium Hypochlorite (bleach) solution.

Note

Cleaning the sample probe using a high-concentration bleach solution (5% or higher) may cause rusting or discoloration. Use normal concentration, aqueous solution.

1 In SH800 Software, click [Bleach Cleaning] on the [Cytometer] tab of the ribbon.

Note

The bleach cleaning sequence must be followed to completion once it is started.

2 Load a 15 ml conical sample tube containing 10 ml of 1 to 3% Sodium Hypochlorite (bleach) solution in the sample loader.

Tip

When performing bleach cleaning during measurement, perform the cleaning sequence once using a 15 ml conical sample tube. When performing bleach cleaning before shutdown at the end of a day, perform the cleaning sequence three times using a 15 ml conical sample tube or once using a 30 ml cleaning tube.

3 Click [Start] to begin the cleaning process.

The solution passes through the sample probe, sample line, and sorting chip.
A progress bar is displayed during cleaning.
Abort the process, if necessary, before the level of bleach in the sample tube falls below the tip of the sample probe.

4 When finished, remove the tube.

5 Click [DI Rinse] on the [Cytometer] tab of the ribbon.

6 Load a 15 ml conical sample tube containing 12 ml of DI water in the sample loader.

The level of DI water must exceed the level of bleach added in step **2**.

7 Click [Start] to begin the rinse process.

Stop the DI rinse process, if necessary, before the level in the sample tube falls below the tip of the sample probe.

8 When finished, remove the tube.

9 Click [Probe Wash] on the [Cytometer] tab of the ribbon to flush the sample line and probe.

Note

Always use 15 ml conical tubes with the specified fluid volume when using the Bleach Cleaning function. If the volume of fluid is insufficient, the cleaning fluids may be ejected from the sorting chip in a spray, contaminating the optical lenses and other components.

General Cleaning

The sorting area and sample loader should be cleaned regularly to prevent the precipitation of salts from the sheath fluid and to prevent the spread of biological matter. Cleaning the instrument regularly also prevents trouble in the cultivation process after sorting.

Clean the droplet camera window, laser windows, collection stage, collection tube holders, and sample loader using water and ethanol.

Notes

- Be aware of the door edges when doors are open.
- Always wear gloves and other protective clothing, mask, and goggles.

1 Turn on the power supply using the POWER/STANDBY button on the front panel.

2 Press the OPEN/CLOSE sample loader door button on the front panel to access the sample loader.

3 Press the PUSH OPEN sorting area door button on the front panel to access the sorting area.

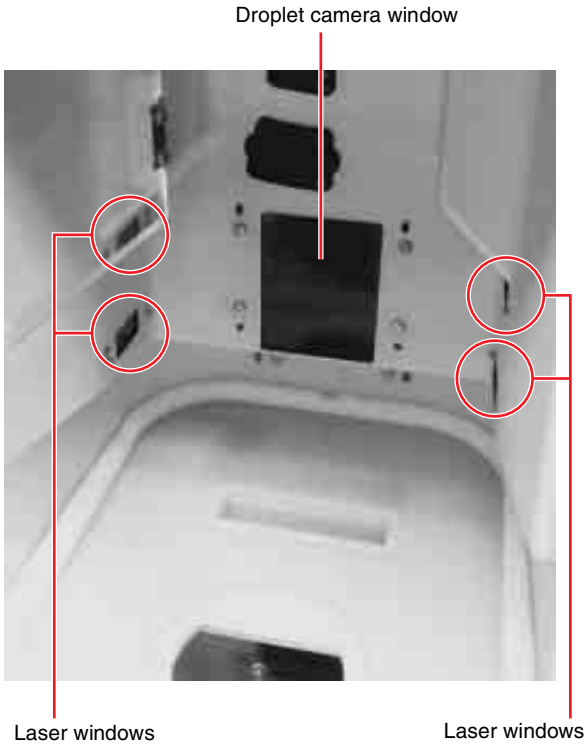
4 Moisten a clean, soft, lint-free laboratory-use cloth with clean water, and wipe with the cloth.

Droplet camera window and laser windows

Clean the droplet camera window to prevent reflections entering the camera, and the laser windows to ensure the detection of the stream position. If the windows become dirty, an error may occur during automatic calibration.

Tip

The number of laser windows varies depending on the model.



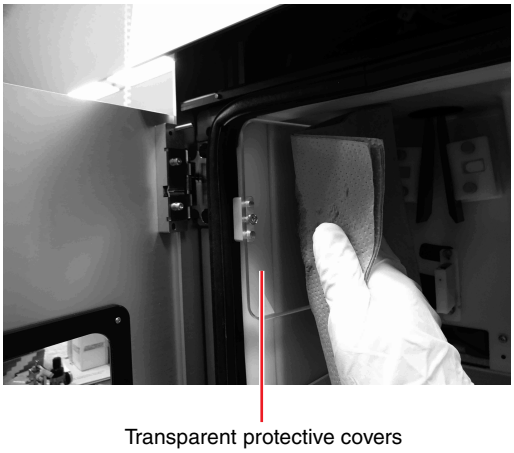
Collection stage and collection tube holders

Clean the collection stage and collection tube holders carefully, especially the upper portion of the collection stage, where the unit is most exposed to salts and biological matter.

Transparent protective covers in the sorting area

Wipe the inner surfaces of the transparent protective covers.

Moisten a clean, soft, lint-free cloth with water, and wipe the covers.



Note

Wiping with a dry cloth can generate static electricity, which may adversely affect the side streams during auto calibration. Never use a dry cloth.

Sample loader

Clean carefully to remove all salt products.

1 Spray a clean, soft, lint-free laboratory-use cloth with ethanol 70% from a spray bottle, and wipe with the cloth.

2 Moisten a clean, soft, lint-free laboratory-use cloth with clean water, and wipe the sample tube loader.

Notes

- Do not operate any hardware or software controls while cleaning the sample loader or the sorting area.
- When cleaning the sample loader, remove the sample tube holder and clean separately.
- When cleaning the sorting area, pull the collection stage out to its maximum travel and remove the collection tube holder and clean separately.
- When cleaning the sorting area, also remove the deflection plates *(page 155)* and waste catcher *(page 157)*, and clean separately.
- Check the drip tray in the main unit and fluidics cart for leaks and spills regularly.

Cleaning the splash guard

1 Moisten a soft, lint-free laboratory use cloth with water and wipe the splash guard.

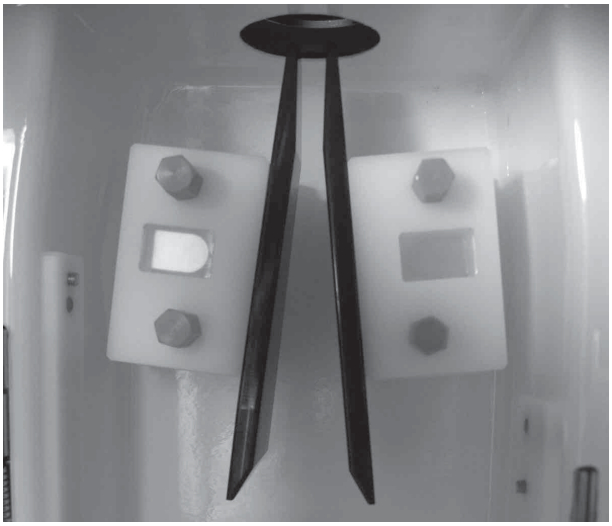
Take care to clean right into the edges and corners.

2 Spray a clean, soft, lint-free cloth with ethanol 70% from a spray bottle, and wipe the guard.

3 Moisten a clean, soft, lint-free cloth with water, and wipe the guard.

Cleaning the Deflection Plates

The deflection plates in the sorting area should be removed and cleaned when cleaning the sorting area to ensure the area is not contaminated between sorts.



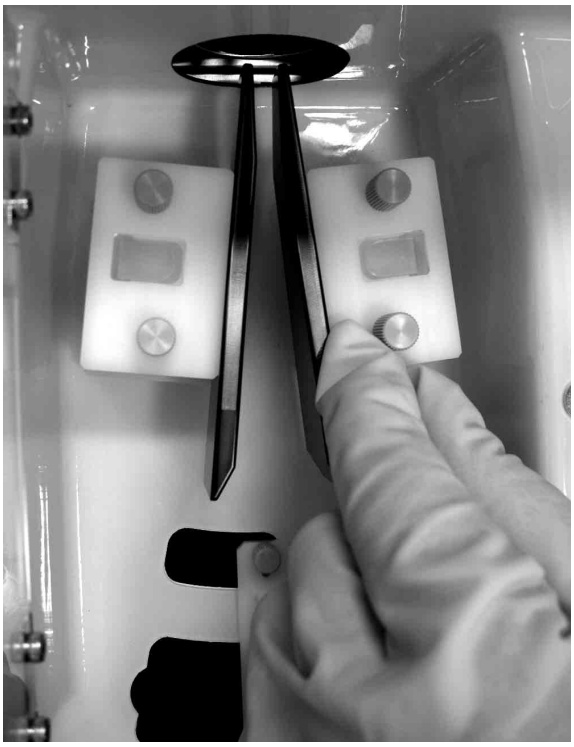
1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel.

2 Open the sorting area door on the front panel.

3 Pull open the transparent safety cover to access the deflection plates.

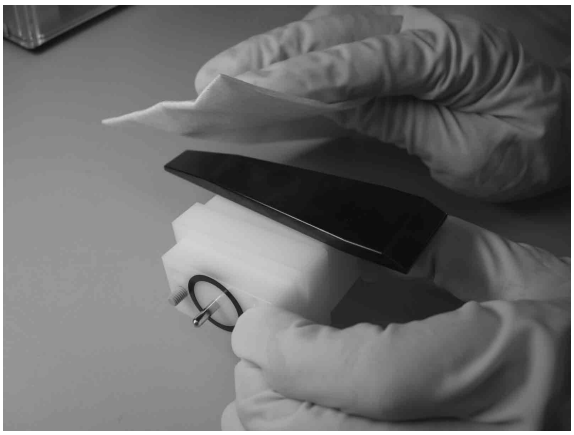
The cover is held closed by a magnetic latch.

4 Remove the two plastic bolts securing each deflection plate by hand, and remove the deflection plates.



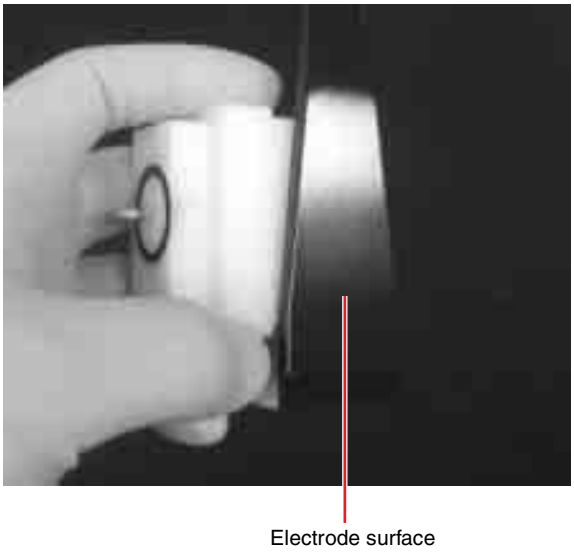
5 Clean the deflection plate mounting plate using a lint-free laboratory-use cloth moistened with water.

6 Clean the deflection plate.



Note

The electrode surfaces of the deflection plates have been treated with a water-repellent coating. Wiping salt crystal deposits with a cloth may damage the coating. Wash the electrode surfaces with water to dissolve salt crystals, then wipe clean.



7 Clean the deflection plate plastic bolts.

8 Spray a lint-free laboratory-use cloth with ethanol and clean the items in steps **5** to **7**.

9 Insert the deflection plate in the correct orientation.

10 Insert the two plastic bolts and tighten by hand.

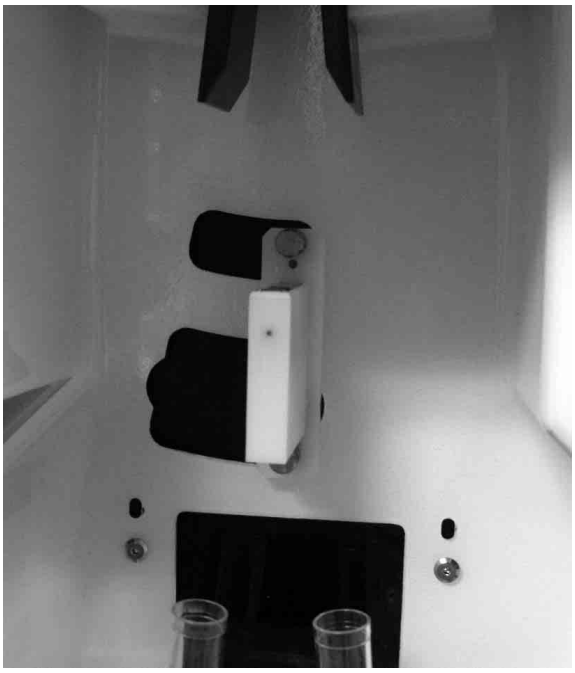
Note

Do not overtighten using a wrench.

11 Close the transparent safety cover.

Cleaning the Waste Catcher

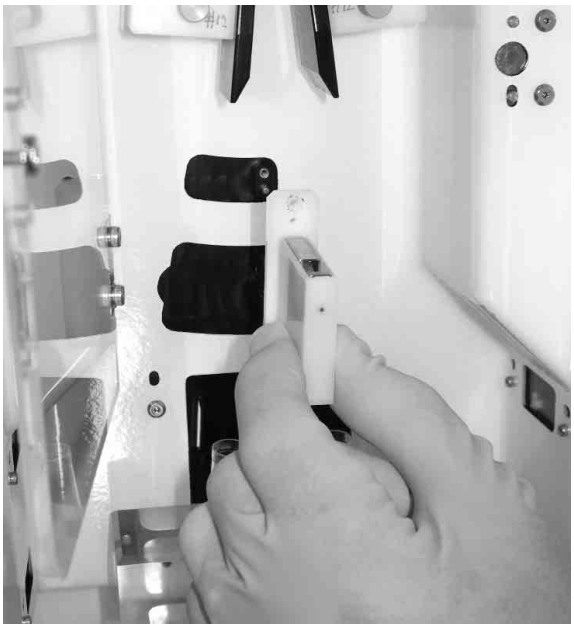
The waste catcher in the sorting area should be removed and cleaned when cleaning the sorting area to ensure the area is not contaminated between sorts.



1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel.

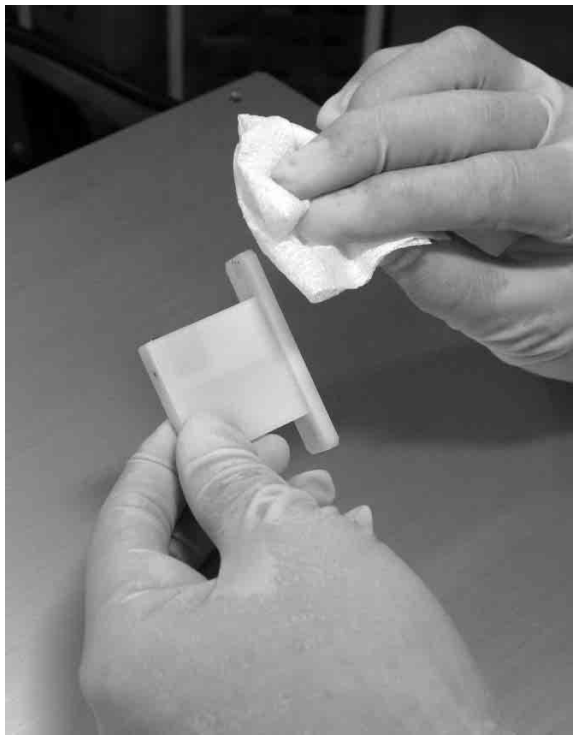
2 Open the sorting area door on the front panel.

3 Remove the two screws securing the waste catcher, and remove the catcher.



4 Clean the waste catcher mounting bracket, holes, and screws using a lint-free laboratory-use cloth moistened with water.

5 Clean the waste catcher.



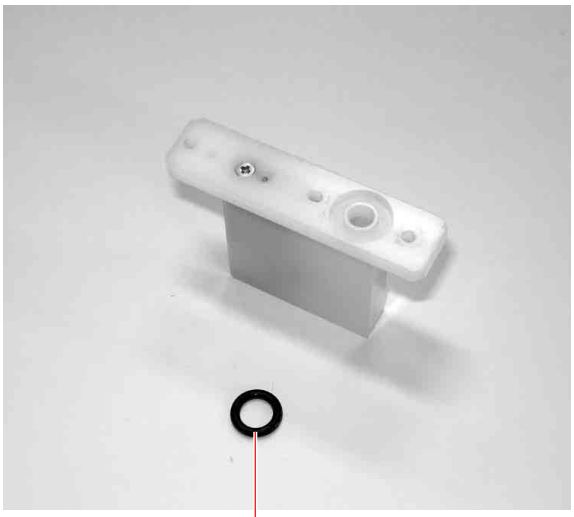
6 Spray a lint-free laboratory-use cloth with ethanol and clean the items in steps **4** and **5**.

Wipe any moisture off the screws using a dry, lint-free laboratory-use cloth.

7 Reinstall the waste catcher.

Notes

- Electrostatic charge may accumulate in the sorting area after cleaning the plastic covers with a cloth that is too dry. This charge can adversely affect the side stream position, causing an error in the automatic side stream calibration process. If this occurs, wipe the areas around the deflection plates with a cloth soaked in DI water to dissipate any accumulated electrostatic charge.
- Make sure the waste fluid outlet and O-ring of the waste catcher are correctly aligned with the port on the mounting plate, and the waste catcher is securely fastened to the mounting plate. There is a risk of waste fluid leakage if not fitted correctly (especially, the O-ring), or risk of overflow if the port is obstructed.
- The O-ring on the rear of the waste catcher must be replaced every 12 months.
- Check that the waste catcher screw is not wet when tightening the screw. A malfunction may occur if the screw is wet when installed.



Running Maintenance Mode

The following maintenance can be performed in maintenance mode.

- Ethanol cleaning
- Waste A maintenance (bleach cleaning)
- Changing the sample line

You can run maintenance mode from the SH800 Software login screen.

Running ethanol cleaning

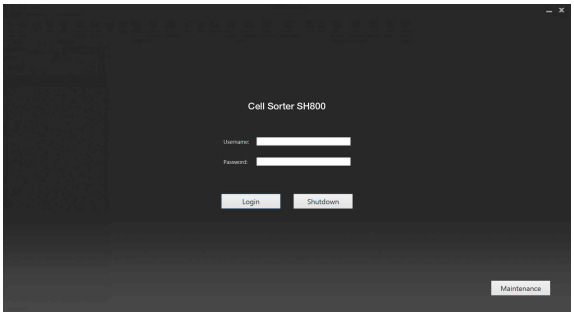
Precautions when cleaning with ethanol

- Ensure the laboratory is well ventilated during cleaning with ethanol. Inhaling ethanol vapor can cause irritation of the eyes, skin and respiratory tract, loss of coordination, drowsiness, and in sufficient concentration, unconsciousness.
- Never place a naked flame, embers, or other material that can emit sparks near the main unit or the fluidics cart. Ethanol vapor is highly flammable at normal room temperature.
- Do not pour any fluid other than the specified fluid into the fluidics system tanks.
- Make sure that the cap on the ethanol tank is correctly and securely fastened at all times.
- Make sure that the ethanol line is securely connected to the connectors on the rear panel of the main unit, rear panel of the fluidics cart, and the ethanol tank itself.
- Make sure that the cap on the waste tank is correctly and securely fastened at all times.
- Make sure that the waste fluid line is securely connected to the connectors on the rear panel of the main unit, rear panel of the fluidics cart, and the waste tank itself.

Note

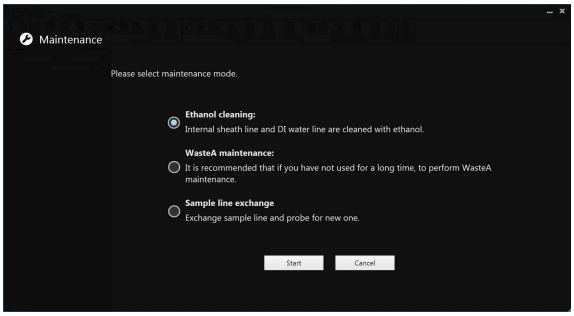
The SH800 is compatible with standard disinfecting agents (ethanol 70%) for use in flow cytometers.

1 Click [Maintenance] on the bottom right of the SH800 Software login screen.



The [Maintenance] wizard appears.

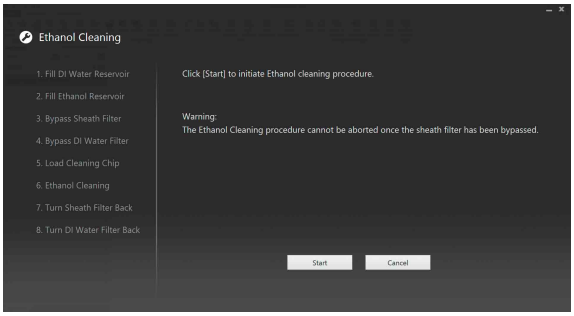
2 Select [Ethanol cleaning], then click [Start].


Maintenance
Please select maintenance mode.
Ethanol cleaning:
Internal sheath line and DI water line are cleaned with ethanol.
WasteA maintenance:
It is recommended that if you have not used for a long time, to perform WasteA maintenance.
Sample line exchange
Exchange sample line and probe for new one.
Start
Cancel

The [Ethanol Cleaning] wizard appears.

3 Click [Start] to begin ethanol cleaning.

Follow the on-screen instructions.


Ethanol Cleaning
1. Fill DI Water Reservoir
2. Fill Ethanol Reservoir
3. Bypass Sheath Filter
4. Bypass DI Water Filter
5. Load Cleaning Chip
6. Ethanol Cleaning
7. Turn Sheath Filter Back
8. Turn DI Water Filter Back
Click [Start] to initiate Ethanol cleaning procedure.
Warning:
The Ethanol Cleaning procedure cannot be aborted once the sheath filter has been bypassed.
Start
Cancel

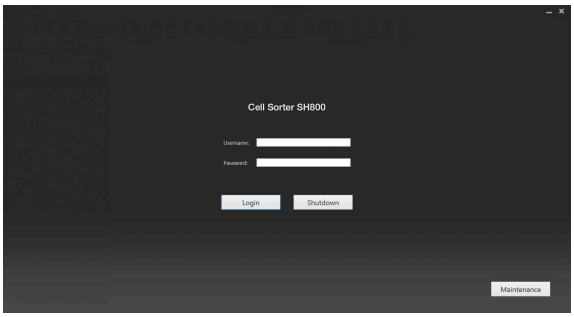
For details about ethanol cleaning, see "Cleaning the Internal Sheath Line and DI Water Line using Ethanol" (page 188).

When ethanol cleaning finishes, the login screen reappears.

Running Waste Line A Maintenance

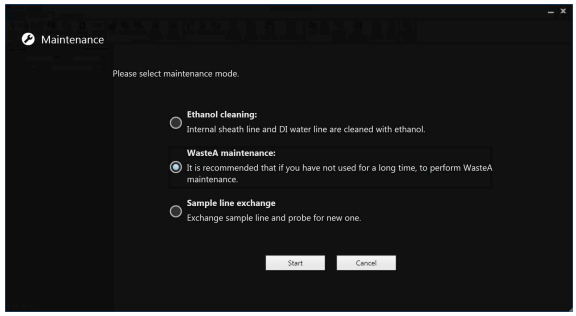
The instrument should be started in maintenance mode to prevent the buildup of salts and clean waste lines (Waste Line A).

1 Click [Maintenance] on the bottom right of the SH800 Software login screen.



The [Maintenance] wizard appears.

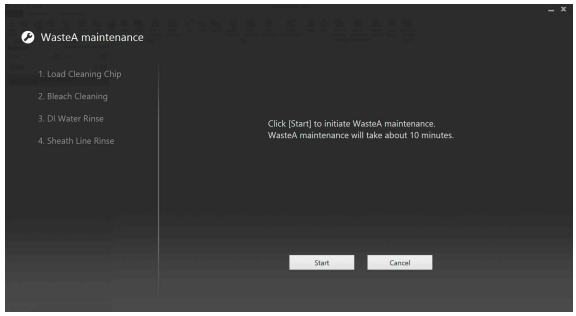
2 Select [WasteA maintenance], then click [Start].


Maintenance
Please select maintenance mode.
Ethanol cleaning:
Internal sheath line and DI water line are cleaned with ethanol.
WasteA maintenance:
It is recommended that if you have not used for a long time, to perform WasteA maintenance.
Sample line exchange
Exchange sample line and probe for new one.
Start
Cancel

The [WasteA maintenance] wizard appears.

3 Click [Start] to begin WasteA maintenance.

Follow the on-screen instructions.
In WasteA maintenance, the fluidics system is cleaned using a sodium hypochlorite solution (bleach cleaning), the DI water lines are cleaned, and the internal sheath lines are cleaned, in that order.

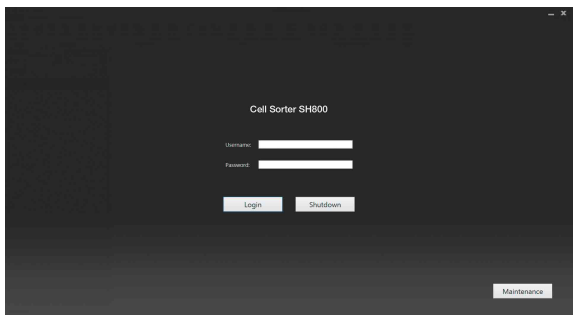


For details about cleaning using Sodium Hypochlorite solution, see "Cleaning the Sample Fluidics System using Bleach" (page 152).

When WasteA maintenance finishes, the login screen reappears.

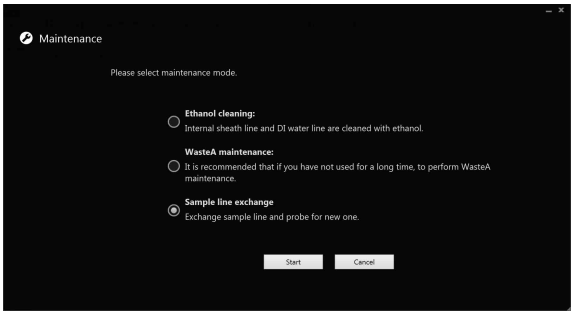
Changing the Sample Line

1 Click [Maintenance] on the bottom right of the SH800 Software login screen.



The [Maintenance] wizard appears.

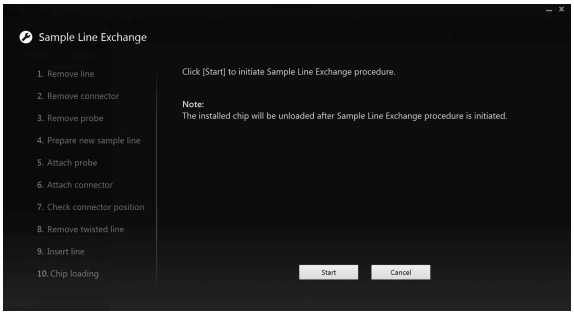
2 Select [Sample line exchange], then click [Start].


Maintenance
Please select maintenance mode.
Ethanol cleaning:
Internal sheath line and DI water line are cleaned with ethanol.
WasteA maintenance:
It is recommended that if you have not used for a long time, to perform WasteA maintenance.
Sample line exchange
Exchange sample line and probe for new one.
Start
Cancel

The [Sample Line Exchange] wizard appears.

3 Click [Start] to begin changing the sample line.

Follow the on-screen instructions.



For details about changing the sample line, see "Changing the PEEK Sample Line (PEEK sample line compatible models)" (page 169) or "Changing the Sample Line and Probe (Non-PEEK sample line compatible models)" (page 172).

When the sample line has been changed, the login screen reappears.

Changing the Sample Loader O-Ring

The sample loader is fitted with an O-ring in the base of the injection chamber that seals the chamber when the sample tube is lifted into the chamber. The O-ring should be cleaned regularly, and replaced every 3 to 6 months to ensure the integrity of the seal.

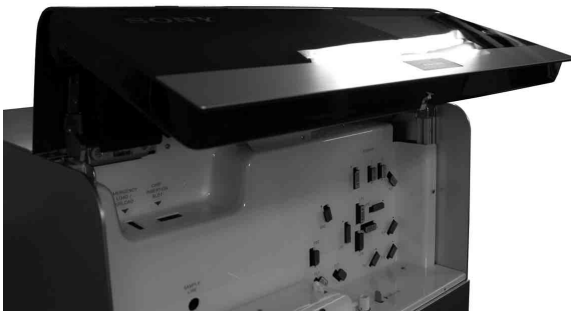
1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel.

2 Open the flip-up door on the front panel to access the sample probe, and hold it in position.

3 Turn off the compressed air supply.

Have an assistant help if you cannot reach the air supply control valve while holding the flip-up door open.

4 Lower the flip-up door slowly until it can support its own weight.



5 Remove the sample probe.

The sample probe is a precision component. It should be removed so that it cannot be damaged accidentally while removing the O-ring. For details, see *"Changing the PEEK Sample Line (PEEK sample line compatible models)" (page 169)* or *"Changing the Sample Line and Probe (Non-PEEK sample line compatible models)" (page 172)*.

6 Push the bottom of the sample loader door in slightly, and then raise the door and hold it open.

If a sample tube is currently in the sample loader, remove it.

7 Push the sample loader waste catcher (white) into its recess to provide access to the bottom of the injection chamber.

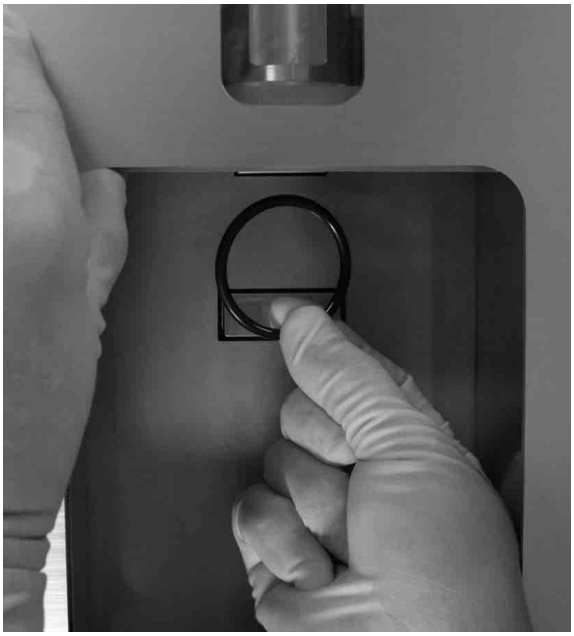
8 Pry out the O-ring using a small flat-blade screwdriver or a pair of tweezers.

Note

Take care not to damage the O-ring, if removing it for cleaning, or the O-ring groove.

9 Withdraw the O-ring.

When cleaning the O-ring, use a soft laboratory-use cloth sprayed with ethanol, and then wipe dry. Also, clean the groove in the sample injection chamber and the top surface and circumference of the sample tube agitation unit that forms the seal with the O-ring.



10 Insert a new or cleaned O-ring into the O-ring groove, and run your finger around the O-ring to ensure it is seated properly.

11 Lower the sample loader door and reinsert the sample probe.

12 Turn on the compressed air supply.

13 Close the flip-up door.

14 Turn on the main power supply.

The sample loader waste catcher will pop back out by itself.

Cleaning the Sheath Tank and Sheath Line

The sheath tank and sheath line should be cleaned regularly with ethanol to suppress the growth of bacteria.

Precautions when cleaning with ethanol

- Ensure the laboratory is well ventilated during cleaning with ethanol. Inhaling ethanol vapor can cause irritation of the eyes, skin and respiratory tract, loss of coordination, drowsiness, and in sufficient concentration, unconsciousness.
- Never place a naked flame, embers, or other material that can emit sparks near the main unit or the fluidics cart. Ethanol vapor is highly flammable at normal room temperature.
- Never pour ethanol into a tank other than the ethanol tank, and conversely, never pour other liquids into the ethanol tank.
- Make sure that the cap on the ethanol tank is correctly and securely fastened at all times.
- Make sure that the ethanol line is securely connected to the connectors on the rear panel of the main unit, rear panel of the fluidics cart, and the ethanol tank itself.
- Make sure that the lid on the waste tank is correctly and securely fastened at all times.
- Make sure that the waste fluid line is securely connected to the connectors on the rear panel of the main unit, rear panel of the fluidics cart, and the waste tank itself.

Note

The SH800 is compatible with standard disinfecting agents (ethanol 70%) used for flow cytometers.

1 Replace and empty the waste tank.

For details, see "Emptying/Changing the Waste Tank" (page 148).

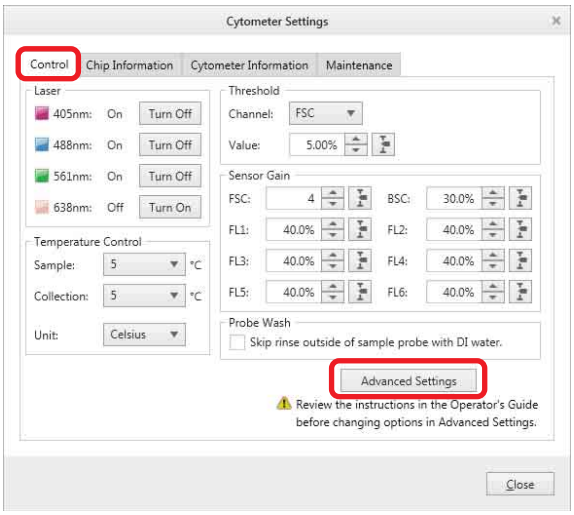
2 Launch SH800 Software.

On the Auto Calibration screen, click [Skip Auto Calibration] to bypass automatic calibration.

3 Click [Settings] on the [Cytometer] tab of the ribbon.

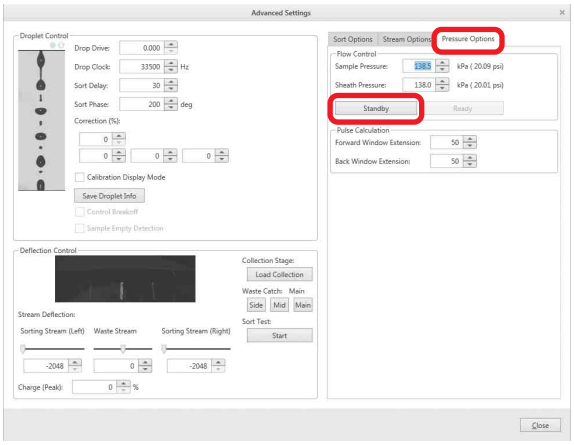
The [Cytometer Settings] dialog appears.

4 On the [Control] tab, click [Advanced Settings].



The [Advanced Settings] dialog appears.

5 On the [Pressure Options] tab, click [Standby] to stop the sheath fluid flow.



6 Perform steps **3** to **8** in *"Refilling the Sheath Tank" (page 146)* to remove the lid of the sheath tank.

7 Dispose of the remaining sheath fluid in the tank.

Note

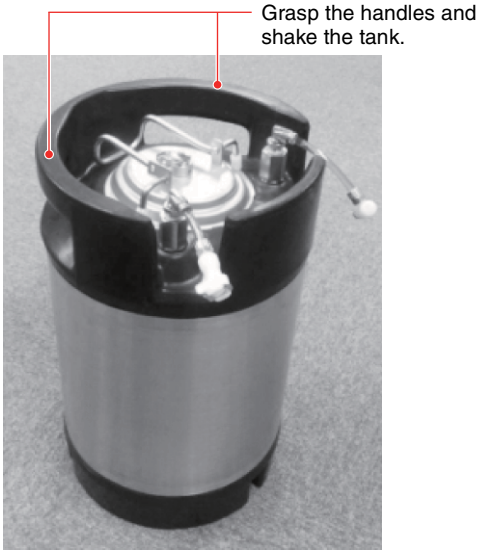
Dispose of the sheath fluid in accordance with the laboratory rules and local ordinances and regulations.

8 Pour 3 liters (0.8 gallons US) of ethanol into the sheath tank and reattach the lid.

Notes

- Do not touch the inner surface of the lid of the sheath tank. Also, take care not to spill any ethanol.
- Do not use a pump or other object that has not been sterilized for pouring ethanol into the tank.
- When inserting the sheath tank lid, make sure that the black rubber seal is attached to the lid.

9 Grasp the handles and shake the sheath tank to clean the inside of the tank (if necessary).



10 Place the tank in the cart tray, and connect the sheath fluid line (blue) and sheath air line (clear) in the reverse order of removal, until they lock into place.

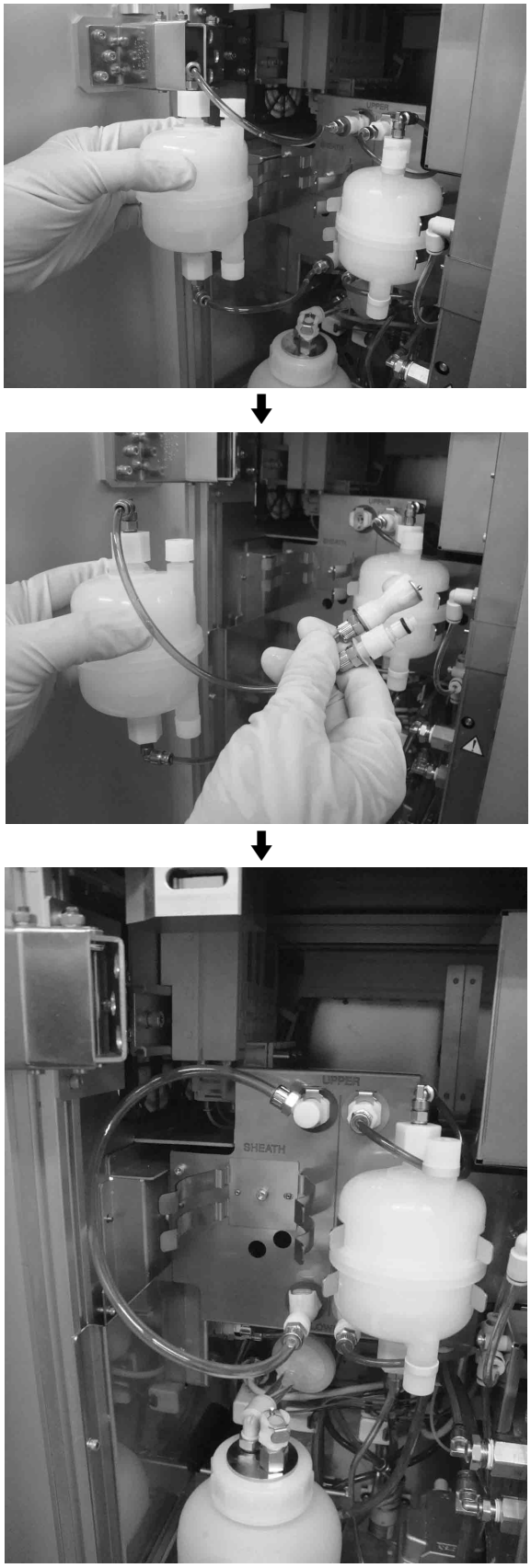
Note

Always connect the sheath fluid line (blue) and sheath air line (clear) in that order. Connecting the sheath air line first will pressurize the sheath tank, causing ethanol to seep out of the sheath fluid line.

11 Clean any fluid from the connectors and outside surfaces of the tank and stow the tray.

Insert the line connector and push until it locks into place. Clean any fluid from the connector and outside surfaces of the tank.

12 Disconnect the sheath filter lines in the main unit, and insert the filter bypass line.



13 In SH800 Software, click [Sheath Filter] in the [De-bubble] group on the [Cytometer] tab of the ribbon.

A popup window appears.

14 Click [Start] to begin cleaning, then click [Close] when finished.

15 Click [Ethanol Cleaning] in the [Shutdown] group on the [Cytometer] tab of the ribbon.

Follow the on-screen instructions to clean the instrument with ethanol.
When cleaning is completed, both the main unit and SH800 Software automatically shut down.

Note

A prompt to reconnect the sheath filter appears on the Ethanol Cleaning screen. However, when cleaning the sheath tank and sheath line, leave the bypass line in place and proceed to the next step.

16 Dispose of the ethanol in the tank, using the same procedure in steps **5** and **6**.

Note

Dispose of the ethanol in accordance with the laboratory rules and local ordinances and regulations.

17 Pour 4 liters (1.1 gallons US) of DI water into the sheath tank and reattach the lid.

Notes

- Do not touch the inner surface of the lid of the sheath tank. Also, take care not to spill any DI water.
- Do not use a pump or other object that has not been sterilized for pouring DI water into the tank.
- When inserting the sheath tank lid, make sure that the black rubber seal is attached to the lid.

18 Stow the sheath tank in the tray using the same procedure as in steps **9** and **10**.

19 Turn on the POWER/STANDBY button on the front panel of the main unit.

20 Launch SH800 Software.

On the Auto Calibration screen, click [Skip Auto Calibration] to bypass automatic calibration.

21 Click [Sheath Filter] in the [De-bubble] group on the [Cytometer] tab of the ribbon.

A popup window appears.

22 Click [Start] to begin cleaning, then click [Close] when finished.

Repeat this procedure at least five times to remove all traces of ethanol in the sheath fluid line.

23 Disconnect the filter bypass line, and reconnect the sheath filter lines.

24 Perform steps **3** to **6** in *"Refilling the Sheath Tank" (page 146)* to remove the sheath tank from the tray.

25 Grasp the handles and shake the sheath tank to clean the inside of the tank, as described in step **8**.

26 Remove the lid of the sheath tank, and dispose of the DI water.

Note

Dispose of the DI water in accordance with the laboratory rules and local ordinances and regulations.

27 Refill the tank with sheath fluid.

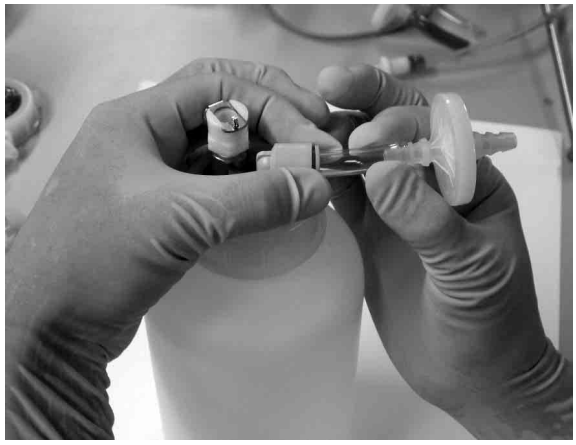
*For details, see step **7** and later in "Refilling the Sheath Tank" (page 147).*

Changing Tank Air Filters

The DI water tank in the main unit, and the ethanol tank and waste tank in the fluidics cart are each fitted with an air filter. The filter allows air to flow into or out of the tank as the fluid level in the tank changes.

The air filters on the three tanks are connected using the same type of connector. This section describes the replacement procedure using the DI water tank as an example.

1 Grasp the short length of tubing with one hand, and push the quick release collet into the connector using your other hand.



2 Pull the tubing out of the connector.

3 Insert the replacement air filter tubing into the connector and push firmly into place.

Note

The filter may become clogged if fluid gets inside the filter, adversely affecting unit operation. If any fluid enters the filter while emptying, replacing, or refilling tanks, replace the filter as soon as possible.

Cleaning the DI Water Tank and DI Water Line

The DI water tank and line should be cleaned regularly with ethanol to suppress the growth of bacteria.

To suppress the growth of bacteria, use sterile distilled water or sterile purified water as the DI water supply for normal use and cleaning.

Precautions when cleaning with ethanol

- Ensure the laboratory is well ventilated during cleaning with ethanol. Inhaling ethanol vapor can cause irritation of the eyes, skin and respiratory tract, loss of coordination, drowsiness, and in sufficient concentration, unconsciousness.
- Never place a naked flame, embers, or other material that can emit sparks near the main unit or the fluidics cart. Ethanol vapor is highly flammable at normal room temperature.
- Do not place any fluid other than the specified fluids in the DI water tank.
- Make sure that the cap on the waste tank is correctly and securely fastened at all times.

Note

The SH800 is compatible with standard disinfecting agents (ethanol 70%) used for flow cytometers.

1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel.

2 Open the fluidics maintenance door, then disconnect the line from the top of the DI water tank.

Press the metal release catch to withdraw the connector.

3 Undo the metal restraining clamp and remove the DI water tank.

Note

Always wear gloves and other protective clothing, mask, and goggles, when handling fluidics system components. Skin cells and hair are the most common cause of bacterial contaminations.

4 Remove the cap, and withdraw the tank probe.

5 Disconnect the quick-release connector from the air filter.

6 Discard any remaining DI water.

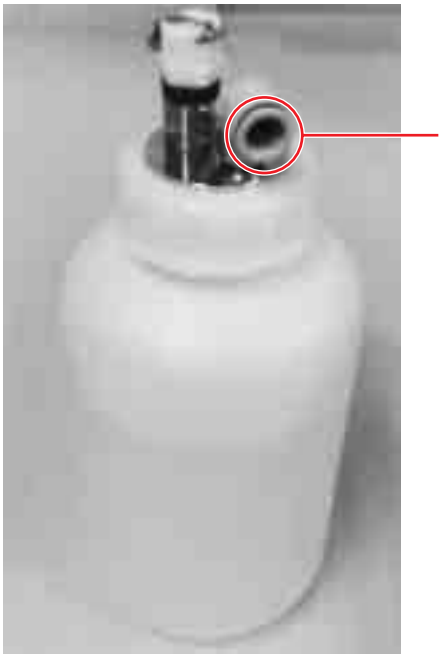
Note

Dispose of DI water in accordance with laboratory rules, and with local and federal ordinances and regulations.

7 Pour 0.5 liters (0.15 gallons US) of ethanol into the DI water tank, and reattach the cap.

8 Shake the tank while holding your finger over the air filter connection port to clean the tank.

9 Remove the DI water filter and connect the DI water filter bypass line (green).



10 Turn on the power supply to the main unit, and start SH800 Software.

11 Click [Probe Wash] on the [Cytometer] tab of the ribbon to wash the sample probe.

Repeat ten times to fill the DI water line with ethanol.

12 Let stand for 20 minutes, then remove the cap of the DI water tank and dispose of the ethanol.

Note

Dispose of ethanol in accordance with laboratory rules, and with local and federal ordinances and regulations.

13 Fill the DI water tank with DI water, then shake the tank with your finger over the air filter connection port to rinse the tank.

14 In SH800 Software, click [Probe Wash] on the [Cytometer] tab of the ribbon to wash the sample probe.

Repeat ten times to remove all traces of ethanol from the DI water line.

15 Remove the cap from the DI water tank and dispose of the DI water.

Note

Dispose of DI water in accordance with laboratory rules, and with local and federal ordinances and regulations.

16 Connect the quick-release connector to the air filter.

17 Refill the tank with fresh DI water up to the opening of the tank, and reattach the cap.

18 Re-install the DI water tank.

Install in the reverse order of removal.

19 Disconnect the DI water filter bypass line (green) and reattach the DI water filter.

20 Check the drip tray for any fluid spills or leakages, and clean as necessary.

21 Close the door, and turn on the power supply.

22 In SH800 Software, click [Probe Wash] on the [Cytometer] tab of the ribbon, as required, to fill the DI water line with DI water.

Changing the PEEK Sample Line (PEEK sample line compatible models)

The PEEK sample line must be replaced regularly to prevent the buildup of salts restricting the sample flow.

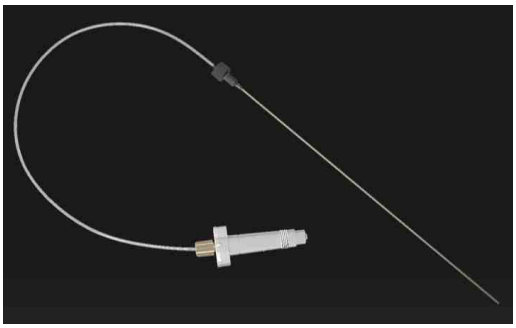
Notes

- The PEEK sample line should be checked for any signs of damage or wear. A damaged PEEK sample line may split and cause sample fluid leakage.
- The PEEK sample line does not support autoclaving (however, the probe adapter does support autoclaving).
- Always leave the PEEK sample line connected when the unit is not in use for protection against dust.
- Use the following procedure to attach the PEEK sample line. There is a risk of fluid leakage if the sample line is not attached correctly.

For details, see "Changing the Probe Adapter (PEEK sample line compatible models)" (page 175).

Tip

Sample fluid may contain biological, chemical, or other agents. Always wear biology laboratory gloves and other protective clothing, mask, and goggles when changing the PEEK sample line.



1 In SH800 Software, click [Sample line] on the [Cytometer] tab of the ribbon.

2 Click [Start] to begin changing the PEEK sample line.

Notes

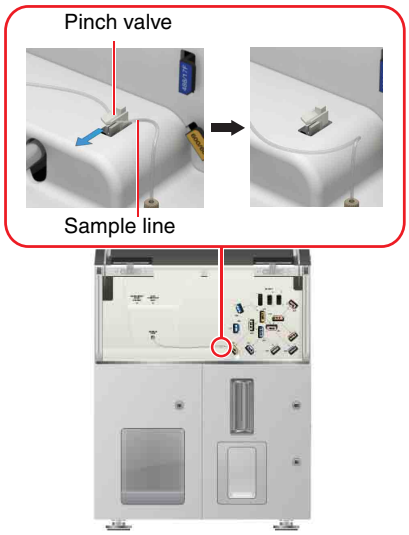
- Always follow the instructions displayed in the wizard when changing the PEEK sample line.
- The sorting chip must be replaced or reinserted after changing the PEEK sample line.

The PEEK sample line can also be changed using [Sample line exchange] on the Initial Instrument Setup screen or using [Sample line exchange] in maintenance mode. For details, see "Logging In" (page 42) and "Running Maintenance Mode" (page 158).

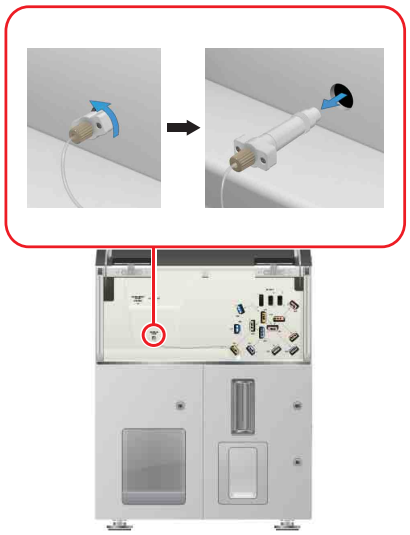
3 Open the flip-up door on the front panel.

4 Remove the sample line from the pinch valve, then click [Next].

To remove, grasp the sample line on both sides of the pinch valve and pull the sample line out towards you.



5 Grasp the T-shaped portion of the sample line connector, and gently unscrew it counterclockwise to remove it from the inlet port on the chip loader, then click [Next].



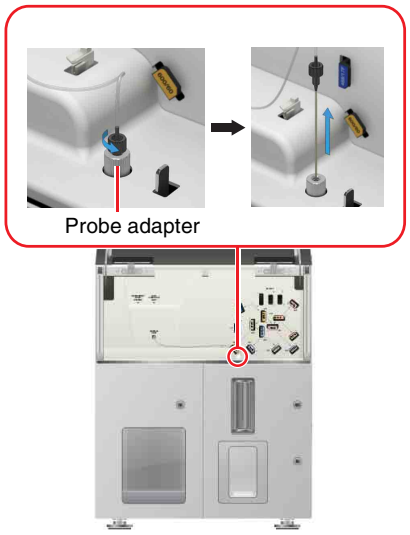
Note

Take care when removing the sample line. Sample fluid may be partially conductive or highly corrosive, which can damage electronic circuits if any droplets fall down between the gaps in the sample loader cover and come into contact with internal components.

6 Grasp the black fitting on the PEEK sample line and gently unscrew it counterclockwise to remove it, then click [Next].

Notes

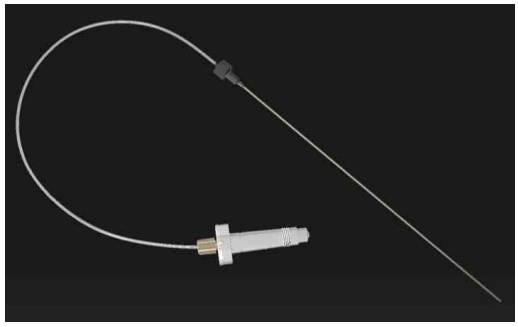
- Unscrew the black fitting while holding the probe adapter down.
- If the sample probe cannot be pulled out, the black fitting may be turning in the reverse direction due to twists in the sample line. Unscrew slowly without releasing the black fitting.



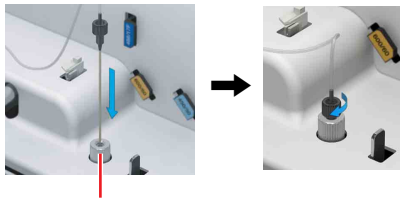
Notes

- Take care when handling the sample probe. It is a high-precision component, which can be damaged easily if handled carelessly.
- The sample line can be discarded. Discard the sample line in accordance with the laboratory rules and local ordinances and regulations.

7 Prepare a new PEEK sample line, then click [Next].



8 Insert the PEEK sample line into the probe adapter, grasp the black fitting, and gently screw it clockwise to secure it, then click [Next].

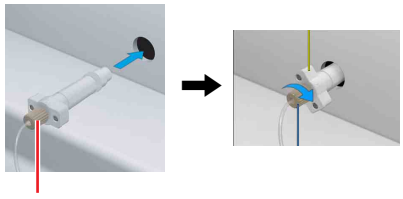


Note

Fully insert and tighten the threaded portion of the fitting. If the fitting is not fully secured, the correct sample pressure may not be applied

9 Attach the new sample line to the inlet port on the front of the chip loader, then click [Next].

Screw part A until it clicks into place, making sure it is securely attached.



Note

When attaching the sample line, always hold part A of the connector, not the T-shaped portion. Holding the connector by the T-shaped portion may cause tightening that exceeds the appropriate tightening torque, and adversely affect instrument performance.

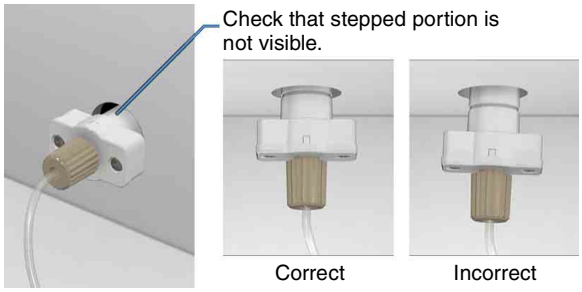
Tips

- When attaching the sample line connector, always screw in the connector until you hear a clicking sound.

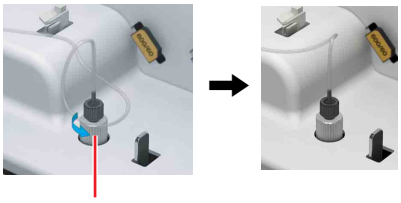
- Check the sample line connector regularly for any signs of looseness.

10 Check that the sample line connector is attached correctly, then click [Next].

When installed correctly, the stepped portion of the sample line connector fits under the cover.
View the connector from the side and check the stepped portion is not visible.

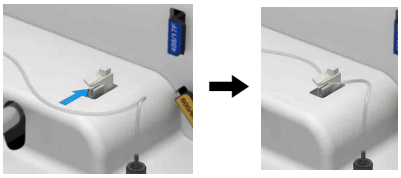


11 Screw in the probe adapter, removing any twists in the sample line, then click [Next].



12 Insert the sample line into the pinch valve, then click [Next].

Make sure the sample line is inserted all the way to the back of the pinch valve.



Notes

- If the sample line is not correctly inserted in the pinch valve, sample fluid may leak from the probe.
- Make sure the sample line connector is securely fastened and that the sample line is correctly inserted in the pinch valve. If the sample line is not inserted correctly, sheath fluid may flow in the reverse direction, potentially causing serious damage to internal components and increasing the risk of biohazard contamination.
- Be sure to insert the sample line into the pinch valve. Failure to do so may result in problems caused by fluid leakage.

13 Click [OK] to exit the [Sample Line Exchange] wizard.

Note

When changing the sample line in maintenance mode, the chip loading screen appears before exiting the [Sample Line Exchange] wizard. Reinsert the unloaded sorting chip, then click [Next].

When the sample line has been changed, the chip exchange screen appears.

Changing the Sample Line and Probe (Non-PEEK sample line compatible models)

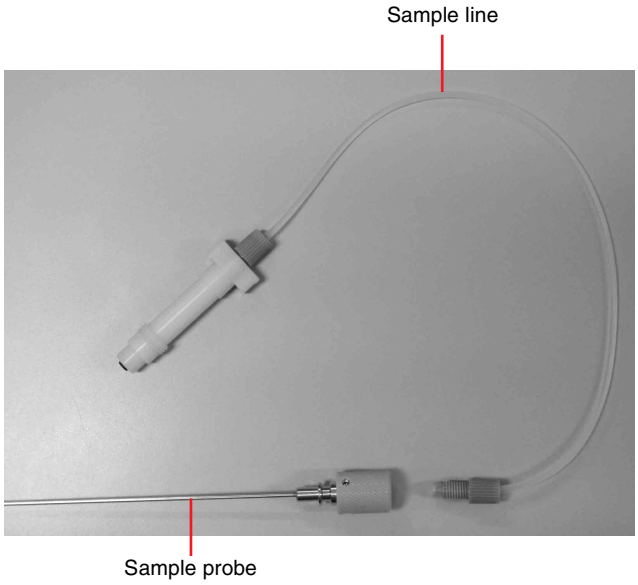
The sample line must be replaced regularly to prevent the buildup of salts restricting the sample fluid flow. The sample line and probe can be removed independently or at the same time. The sample probe can then be detached for autoclaving and then reattached to a replacement sample line for reinstallation.

Notes

- The sample line should be checked regularly for any signs of damage or wear. A damaged sample line may split and cause sample fluid leakage.
- Always leave the sample line connected when the unit is not in use for protection against dust.
- Use the following procedure to attach the sample line. There is a risk of fluid leakage if the sample line is not attached correctly.

WARNING

Sample fluid may contain biological, chemical, or other agents. Always wear gloves and other protective clothing, mask, and goggles, when changing the sample line or sample probe.



1 In SH800 Software, click [Sample line] on the [Cytometer] tab of the ribbon.

2 Click [Start] to begin changing the sample line and sample probe.

Notes

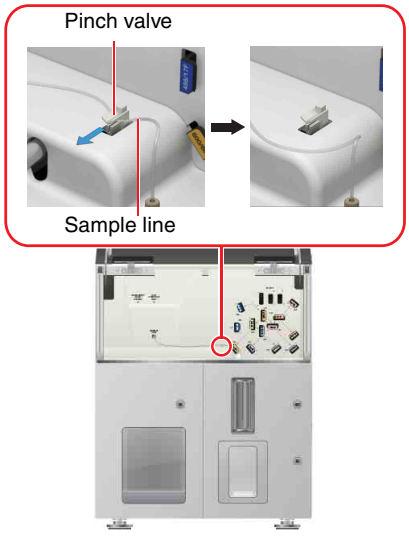
- Always follow the instructions displayed in the wizard when changing the sample line and sample probe.
- The sorting chip must be replaced or reinserted after changing the sample line and sample probe.

The sample line and sample probe can also be changed using [Sample line exchange] on the Initial Instrument Setup screen or using [Sample line exchange] in maintenance mode. For details, see "Logging In" (page 42) and "Running Maintenance Mode" (page 158).

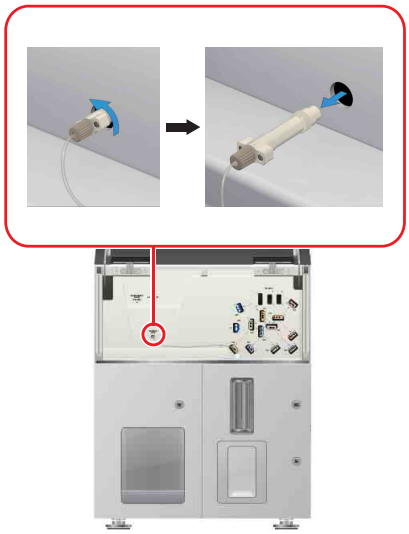
3 Open the flip-up door on the front panel.

4 Remove the sample line from the pinch valve, then click [Next].

To remove, grasp the sample line on both sides of the pinch valve and pull the sample line out towards you.



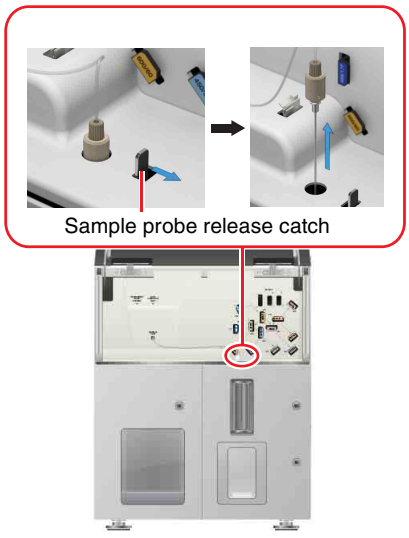
5 Grasp the T-shaped portion of the sample line connector, and gently unscrew it counterclockwise to remove it from the inlet port on the chip loader, then click [Next].



Note

Take care when removing the sample line. Sample fluid may be partially conductive or highly corrosive, which can damage electronic circuits if any droplets fall down between the gaps in the sample loader cover and come into contact with internal components.

6 Push and hold the sample probe release catch to the right, and pull the probe straight up and out, then click [Next].

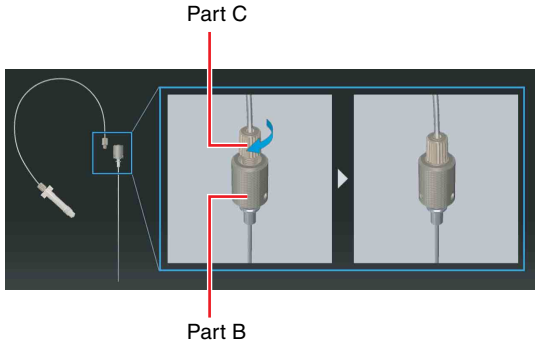


Notes

- Take care when handling the sample probe. It is a high-precision component, which can be damaged easily if handled carelessly.
- The sample line can be discarded. Discard the sample line in accordance with the laboratory rules and local ordinances and regulations.

7 Prepare a new sample line and sample probe, attach the sample line to the sample probe, then click [Next].

Hold part B of the sample probe, and screw part C on the sample line until it clicks into place, making sure it is securely attached.



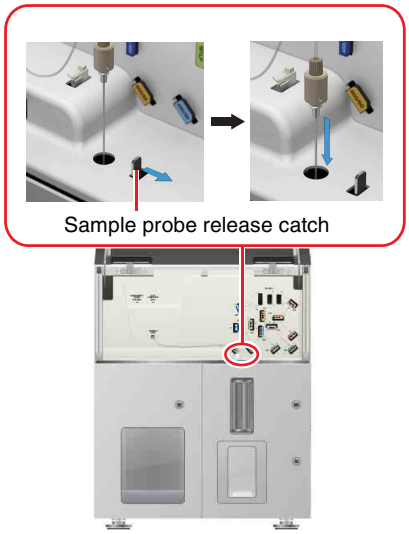
Note

If reattaching the removed sample line and sample probe, disinfect the components using the following procedure, as required.

- Place the sample line in a beaker (or other container) of ethanol and let sit for about 30 minutes.
- Autoclave the sample probe under general autoclave conditions (121 °C (250 °F), 2 atmospheres, approx. 20 minutes).

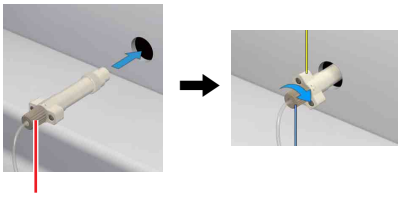
8 Insert the sample probe while pressing its metal release catch to the right.

Press down on the sample probe to insert it securely, and release the metal release catch. Verify that the metal release catch has returned to the left side.



9 Open the flip-up door, and attach the new sample line to the inlet port on the front of the chip loader, then click [Next].

Screw part A until it clicks into place, making sure it is securely attached.



Part A

Note

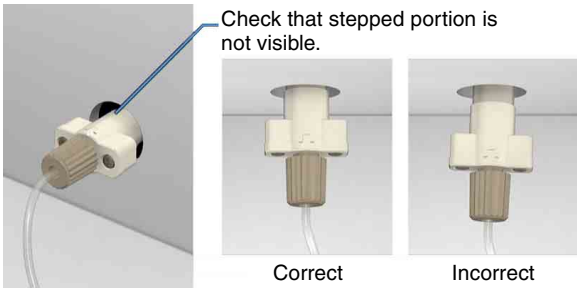
When attaching the sample line, always hold part A of the connector, not the T-shaped portion. Holding the connector by the T-shaped portion may cause tightening that exceeds the appropriate tightening torque, and adversely affect instrument performance.

Tips

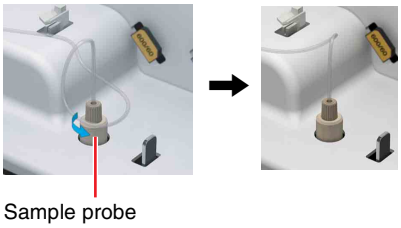
- When attaching the sample line connector, always screw in the connector until you hear a clicking sound.
- Check the sample line connector regularly for any signs of looseness.

10 Check that the sample line connector is attached correctly, then click [Next].

When installed correctly, the stepped portion of the sample line connector fits under the cover.
View the connector from the side and check the stepped portion is not visible.



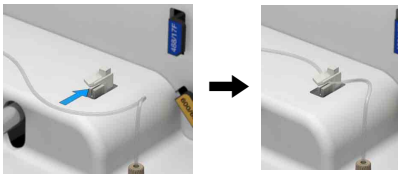
11 Screw in the sample probe, removing any twists in the sample line, then click [Next].



Sample probe

12 Insert the sample line into the pinch valve, then click [Next].

Make sure the sample line is inserted all the way to the back of the pinch valve.



Notes

- If the sample line is not correctly inserted in the pinch valve, sample fluid may leak from the probe.
- Make sure the sample line connector is securely fastened and that the sample line is correctly inserted in the pinch valve. If the sample line is not inserted correctly, sheath fluid may flow in the reverse direction, potentially causing serious damage to internal components and increasing the risk of biohazard contamination.
- Be sure to insert the sample line into the pinch valve. Failure to do so may result in problems caused by fluid leakage.

13 Click [OK] to exit the [Sample Line Exchange] wizard.

Note

When changing the sample line in maintenance mode, the chip loading screen appears before exiting the [Sample Line Exchange] wizard. Reinsert the unloaded sorting chip, then click [Next].

When the sample line has been changed, the chip exchange screen appears.

Changing the Probe Adapter (PEEK sample line compatible models)

The probe adapter that accepts the probe part of the PEEK sample line must be replaced regularly. The probe adapter can be removed, disinfected using autoclaving, and then reinserted back into the unit.

WARNING

Sample fluid may contain biological, chemical, or other agents. Always wear biology laboratory gloves and other protective clothing, mask, and goggles when changing the probe adapter.

Note

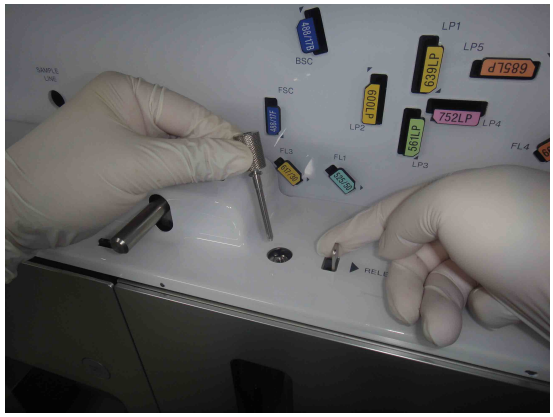
Change the probe adapter using the following procedure after removing the PEEK sample line. In particular, when disinfecting the probe adapter for reuse, always follow the procedure correctly to change the adapter.

1 Remove the PEEK sample line.

*For details about removal, see steps **1** to **6** in "Changing the PEEK Sample Line (PEEK sample line compatible models)" (page 169).*

2 Remove the probe adapter.

Push and hold the sample probe release catch to the right, then pull the probe straight up and out.



3 Disinfect the probe adapter, as required.

Autoclave the probe adapter under general autoclave conditions (121 °C (250 °F), 2 atmospheres, approx. 20 minutes).

Note

Make sure the probe adapter is autoclaved if reusing the probe adapter.

To reinstall

1 Install the probe adapter.

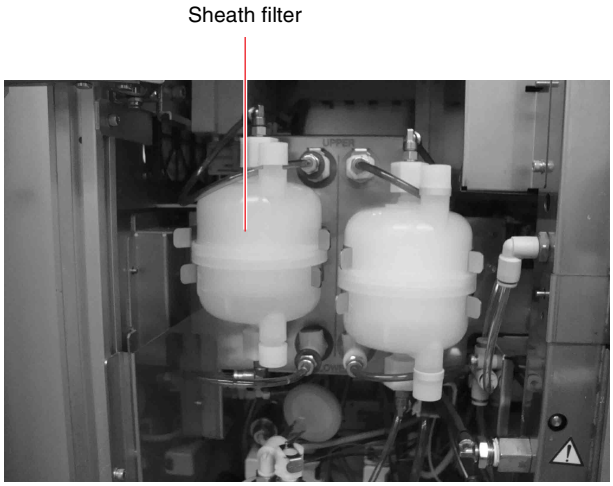
Push and hold the sample probe release catch to the right, insert the probe straight down, and then let go of the release catch. Check that the release catch returns all the way to the left.

2 Install the PEEK sample line.

*For details about installation, see steps **7** to **13** in "Changing the PEEK Sample Line (PEEK sample line compatible models)" (page 169).*

Changing the Sheath Filter

The sheath filter in the fluidics module should be replaced regularly to prevent small particles of dust that can cause fluctuations in the fluid flow and bacteria that can cause contamination of the sample.



This procedure is performed with the power supply turned on and SH800 Software up and running.

1 In SH800 Software, click [Settings] on the [Cytometer] tab of the ribbon.

The [Cytometer Settings] dialog appears.

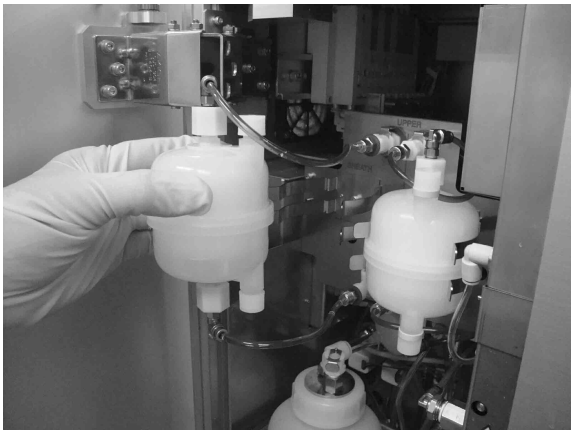
2 Click [Advanced Settings].

The [Advanced Settings] dialog appears.

3 Click [Standby] in the [Pressure Options] tab of the [Advanced Settings] dialog.

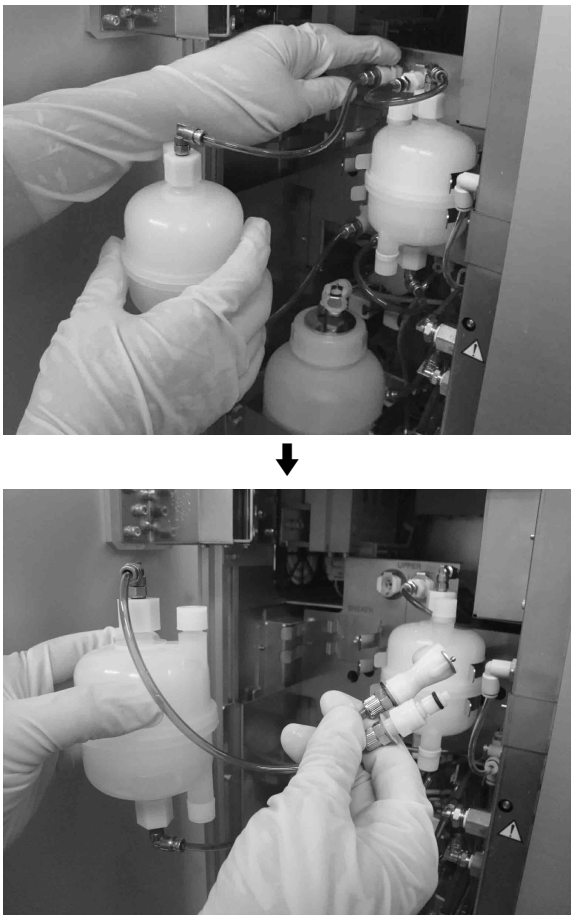
The sheath fluid flow stops.

4 Open the fluidics maintenance door and remove the sheath filter from the retaining clamp without disconnecting the connectors.



5 Disconnect the sheath filter lines from the “UPPER” and “LOWER” connectors.

Press the metal release catch and withdraw each connector. The sheath filter connectors have stop valves that prevent leakage of fluid when disconnected.

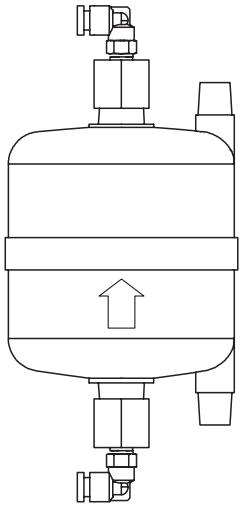


6 Discard the used sheath filter.

Note

Discard the sheath filter responsibly, in accordance with local and federal ordinances and regulations.

7 Hold a new sheath filter with the arrow on the side of the filter facing up.



8 Connect the two fluidics lines from the sheath filter to the corresponding connectors.

Connect the line from the top of the filter to the "UPPER" connector, and the line from the bottom to the "LOWER" connector. Insert each connector and push it until it locks into place. A click sound occurs when the connectors lock into place.

9 Place the sheath filter in the retaining clamp.

10 In SH800 Software, click [Sheath Filter] in the [De-bubble] group on the [Cytometer] tab of the ribbon.

The [Sheath Filter De-bubble] dialog appears.

11 Click [Start]. When the de-bubble function is completed, click [Close]. Repeat two times to remove residual air from the sheath filter.

Note

The sheath filter de-bubble process takes a short time to finish. Run the sheath filter de-bubble function at least three times to make sure that all air and small particles have been removed from the filter and sheath line. Failing to do so may cause any solid matter not removed from accumulating and clogging the sorting chip.

12 Perform steps **4** to **8** as described in *"To release air trapped in the sheath filter manually" (page 181)*.

13 Click [Ready] in the [Pressure Options] tab of the [Advanced Settings] dialog.

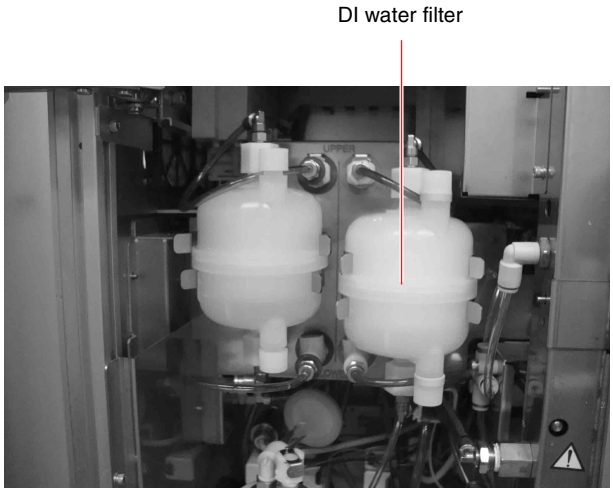
The sheath fluid flow restarts.

14 Load and run a sample tube containing sheath fluid, and check for leaks around the connectors.

Check the drip tray for any fluid spills or leakages, and clean as necessary.

Changing the DI Water Filter

The DI water filter in the fluidics module should be replaced regularly to prevent small particles of dust that can cause fluctuations in the fluid flow and bacteria that can cause contamination of the sample.

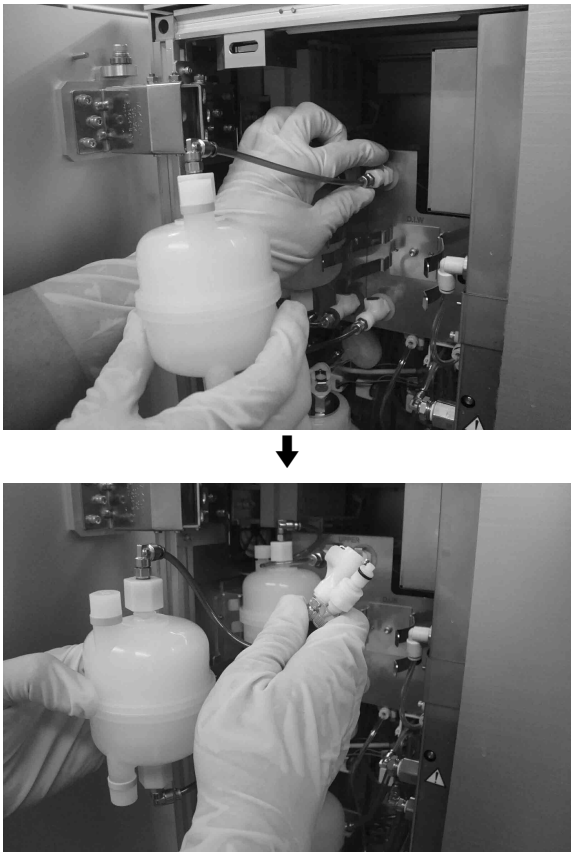


1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel.

2 Open the fluidics maintenance door and remove the DI water filter from the retaining clamp without disconnecting the connectors.

3 Disconnect the DI water filter lines from the "UPPER" and "LOWER" connectors.

Press the metal release catch and withdraw each connector. The DI water filter connectors have stop valves that prevent leakage of fluid when disconnected.

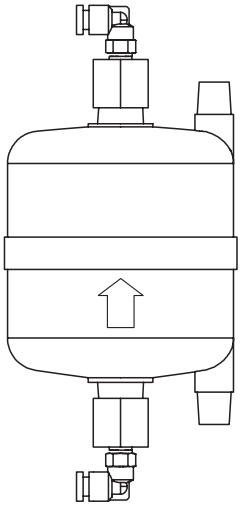


4 Discard the used DI water filter.

Note

Discard the DI water filter responsibly, in accordance with local and federal ordinances and regulations.

5 Hold a new DI water filter with the arrow on the side of the filter facing up.



6 Connect the two fluidics lines from the DI water filter to the corresponding connectors.

Connect the line from the top of the filter to the "UPPER" connector, and the line from the bottom to the "LOWER" connector. Insert each connector and push it until it locks into place. A click sound occurs when the connectors lock into place.

7 Place the DI water filter in the retaining clamp.

8 Close the fluidics maintenance door and turn on the power supply and log in.

9 Open the fluidics maintenance door, remove the DI water filter from the retaining clamp (without disconnecting the connectors), and check the fluid level.

10 Turn on the power supply to the main unit, and start SH800 Software.

11 Click [Settings] on the [Cytometer] tab of the ribbon to display the [Cytometer Settings] dialog. Click [DI Filter de-bubble] on the [Maintenance] tab.

The [DI Filter de-bubble] dialog appears.

12 Click [Start].

13 Release the air trapped in the DI water filter.

Slowly open the air release port on the DI water filter, within five minutes of startup, to release the trapped air.
If air is not released within five minutes after startup, turn the power supply to the main unit off and then on again, then repeat the procedure.

Notes

- It is recommended that the drip tray be pulled out partway before proceeding. Slowly open the air release port while holding a soft, clean cloth ready to wipe away any DI water overflow.
- Keep the DI water filter clear of all other objects when the air release port is open.

The trapped air escapes and the fluid level in the DI water filter begins to rise.

14 Close the air release port when the fluid level nears the top of the filter.

Make sure that the air release port is tightened and that there is no leakage of fluid.

Note

If the air release port is overtightened, the filter may become damaged. Do not apply excess force when tightening.

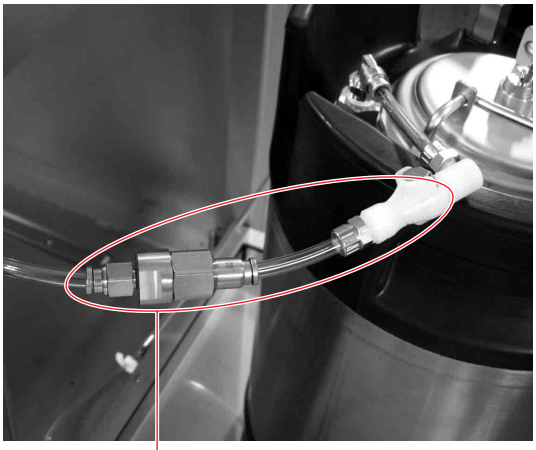
15 Click [Stop].

16 Place the DI water filter in the retaining clamp, and close the fluidics maintenance door.

17 When finished, click [Close].

Changing the Sintered Sheath Line Filter

A sintered sheath line filter is placed in the sheath line to filter residue or small particles from the sheath fluid supply. The filter should be replaced every 12 months.



1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel of the main unit.

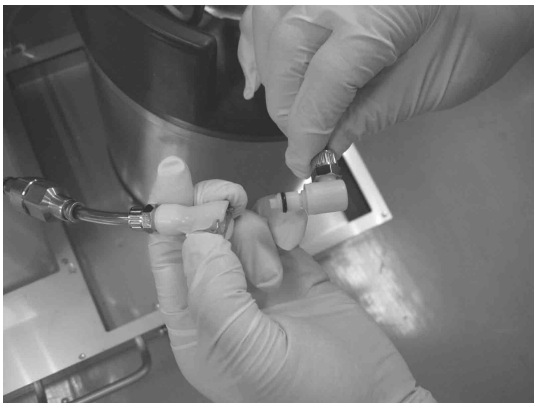
Make sure the main unit is completely shut down before proceeding.

2 Turn off the compressed air supply.

3 Hold the sintered sheath line filter with one hand, push and hold in the quick release collet with the other hand, and pull out the sheath fluid line (blue) from the side furthest from the sheath tank.

Exert gentle force if the tube feels difficult to pull out from the connector.

4 In the same way, remove the white connector on the sheath tank side, and discard the filter.



Note

Discard the sintered sheath line filter in accordance with local and federal ordinances and regulations.

5 Insert a new sintered sheath line filter in the sheath fluid line, pushing the line all the way into the filter, then connect the white connector to the sheath tank.

Releasing Air in the Sheath Filter and Sorting Chip

During operation, air may become trapped in the sheath filter, interrupting the smooth flow of sheath fluid to the sorting chip. Air may also become trapped in the sorting chip itself, preventing the formation of regular-shaped droplets.

To release air trapped in the sheath filter using the de-bubble function

1 Click [Sheath Filter] in the [De-bubble] group on the [Cytometer] tab of the ribbon.

The [Sheath Filter De-bubble] dialog appears.

2 Click [Start], and then click [Close] when finished.

The sheath filter de-bubble process takes a short time to finish.
If changing the sheath filter, run the sheath filter de-bubble process at least three times to make sure that all air and small particles have been removed from the filter and the sheath line.

To release air trapped in the sorting chip using the de-bubble function

If the formation of droplets is irregular even though there is no air trapped in the sheath filter, de-bubble the sorting chip. Click [Chip] in the [De-bubble] group on the [Cytometer] tab of the ribbon.

To release air trapped in the sheath filter manually

If the volume of air trapped in the sheath filter is large, it may be more efficient to release the trapped air manually before using the de-bubble function.

This procedure is performed with the power supply turned on and SH800 Software up and running.

1 In SH800 Software, click [Settings] on the [Cytometer] tab of the ribbon.

The [Cytometer Settings] dialog appears.

2 Click [Advanced Settings].

The [Advanced Settings] dialog appears.

3 Click [Standby] in the [Pressure Options] tab of the [Advanced Settings] dialog.

The sheath fluid flow stops.

4 Open the fluidics maintenance door, remove the sheath filter from the retaining clamp (without disconnecting the connectors), and check the fluid level in the filter.

5 If the fluid level is low, slowly open the air release port on the top of the sheath filter.

Notes

- It is recommended that the drip tray be pulled out partway before proceeding. Slowly open the air release port while holding a soft, clean cloth ready to wipe away any sheath fluid overflow.
- Keep the sheath filter clear of all other objects when the air release port is open.

The trapped air escapes and the fluid level in the sheath filter begins to rise.

6 Close the air release port when the fluid level nears the top of the filter.

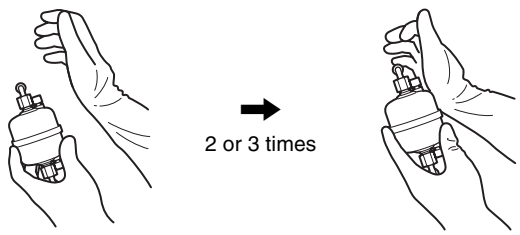
Make sure that the air release port is tightened and that there is no leakage of fluid.

Note

If the air release port is overtightened, the filter may become damaged. Do not apply excess force when tightening.

7 Tap the side of the sheath filter with your hand 2 or 3 times.

This dislodges any trapped air in the lines, which then collects in the sheath filter.



Use the following procedure if there are air bubbles in the sheath filter.

① Open the air release port to expel the trapped air.

② Check that the air has been released and then close the air release port.

③ Tap the filter again and check that there are no air bubbles being formed.

Repeat steps ① to ③ until all air has been released.

8 Place the sheath filter in the retaining clamp, then close the fluidics maintenance door.

9 Click [Ready] in the [Pressure Options] tab of the [Advanced Settings] dialog.

The sheath fluid flow restarts.

Note

Check that there are no air bubbles in the sheath filter before running samples for measurement. It is recommended that you use this procedure if there are any air bubbles in the sheath filter.

Releasing Air in the DI Water Filter

During operation, air may become trapped in the DI water filter, interrupting the smooth flow of DI water.

To release air trapped in the DI water filter using the de-bubble function

1 Click [Settings] on the [Cytometer] tab of the ribbon.

The [Cytometer Settings] dialog appears.

2 Click [DI Water de-bubble] on the [Maintenance] tab

The [DI Water de-bubble] dialog appears.

3 Click [Start].

4 Open the fluidics maintenance door, remove the DI water filter from the retaining clamp (without disconnecting the connectors).

5 Slowly open the air release port on the top of the DI water filter.

Notes

- It is recommended that the drip tray be pulled out partway before proceeding. Slowly open the air release port while holding a soft, clean cloth ready to wipe away any DI water overflow.
- Keep the DI water filter clear of all other objects when the air release port is open.

The trapped air escapes and the fluid level in the DI water filter begins to rise.

6 Close the air release port when the fluid level nears the top of the filter.

Make sure that the air release port is tightened and that there is no leakage of fluid.

Note

If the air release port is overtightened, the filter may become damaged. Do not apply excess force when tightening.

7 Click [Stop].

8 Place the DI water filter in the retaining clamp, and close the fluidics maintenance door.

9 When finished, click [Close].

Autoclaving the Sheath Filter and DI Water Filter

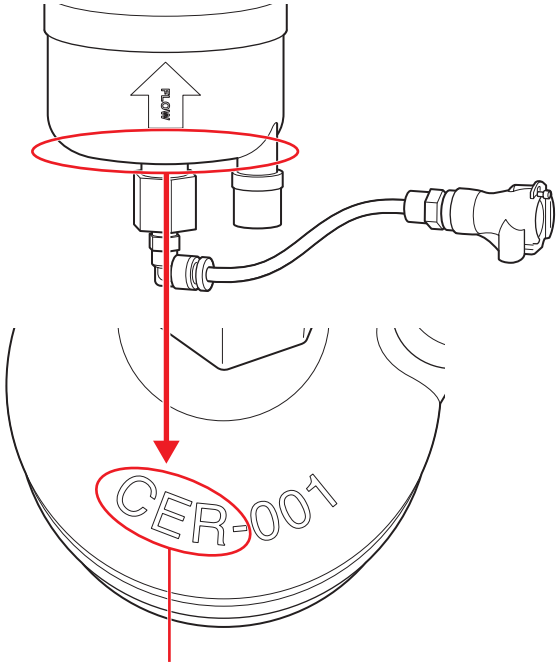
You can autoclave the sheath filter and DI water filter if using autoclavable parts to sterilize the filters.

Notes

- Only the filters are autoclavable.
- Do not autoclave the filters more than once at a time.
- Each filter can be autoclaved up to ten times.

Before autoclaving

Check that the filter has a “CER” marking (autoclavable parts).
The filter marking is on the bottom of the filter body.

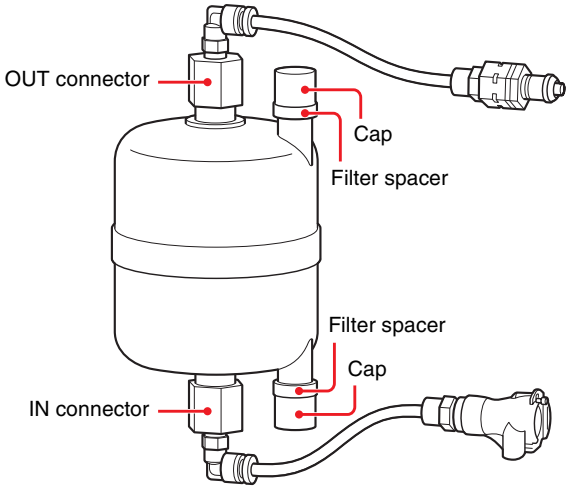


Check the label.
CER: Autoclavable
CSS: Non-autoclavable

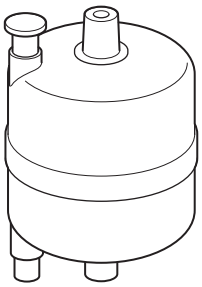
1 Remove the filter from the main unit.

For details about removing filters, see “Changing the Sheath Filter” (page 176) and “Changing the DI Water Filter” (page 177).

2 Unscrew the IN and OUT connectors from the plastic couplers, and remove the cap and filter spacer from the filter.



The filter should have external parts removed.



Note

Do not remove the metallic couplers.

3 Dispose of any fluid inside the filter.

Drain the fluid from the filter until there is no remaining fluid.

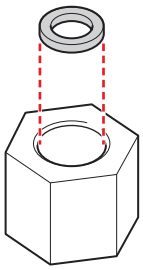
4 Autoclave the filter under the following conditions.

Temperature: 121 °C (250 °F)
Pressure: 2 atmospheres
Duration: 30 minutes

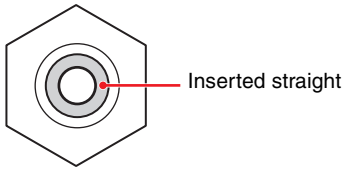
5 After autoclaving, attach the couplers and reinsert into the main unit.

① Attach the IN and OUT connectors.
Tightening torque: 0.50 ± 0.05 N·m

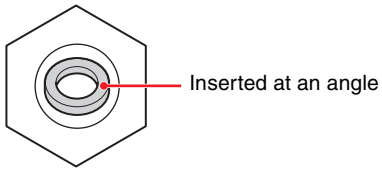
If the cap has a washer, check that the washer is inserted correctly for proper sealing of the cap.



Correct



Incorrect



Note

Attach the IN and OUT connectors to the appropriate ends of the filter.

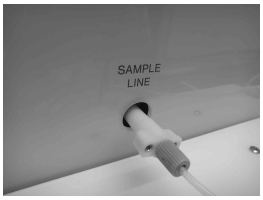
② Attach the filter spacer and cap.
Do not forget to add the filter spacer.

Ejecting the Sorting Chip Manually

The sorting chip is ejected automatically when you click [Unload] in SH800 Software. However, if for any reason the chip will not eject normally, the chip can be removed manually.

1 Open the flip-up door on the front panel to access the sorting chip, and hold it in position.

If the sample line connector is out at the front, go to step **6**.

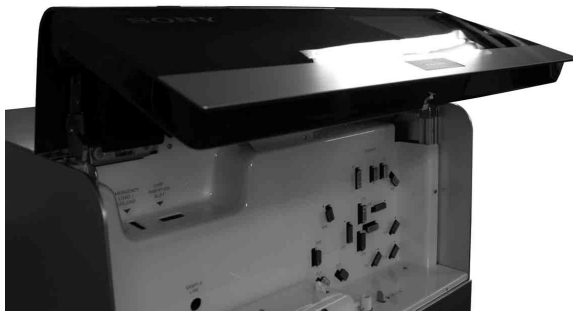


If the sample line connector is not out at the front, go to step **2**.

2 Turn off the compressed air supply.

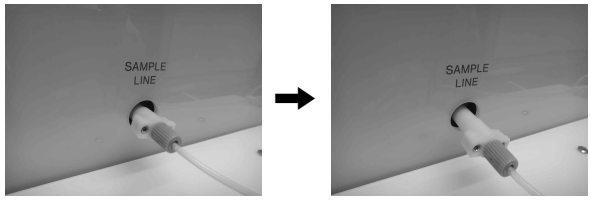
Have an assistant help if you cannot reach the air supply control valve while holding the flip-up door open.

3 Lower the flip-up door slowly until it can support its own weight.



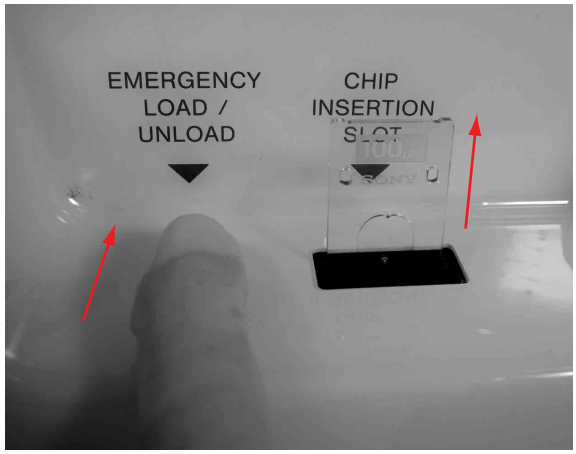
4 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel.

5 Grasp the sample line connector firmly and pull it out towards you to the position, if not in position already.



Pulling the sample line connector withdraws the sorting chip connector interface, allowing the chip to be removed.

6 Eject the chip by pushing the top of the thumbwheel from front to back.



Note

Touching the thumbwheel or the sample line will directly affect the chip alignment. If you touch either and then opt to leave the chip inserted, the chip must be realigned. To align the chip, click [Chip Alignment] on the [Cytometer] tab of the ribbon.

7 When finished, turn on the compressed air supply.

8 Close the flip-up door.

9 Turn on the main power supply.

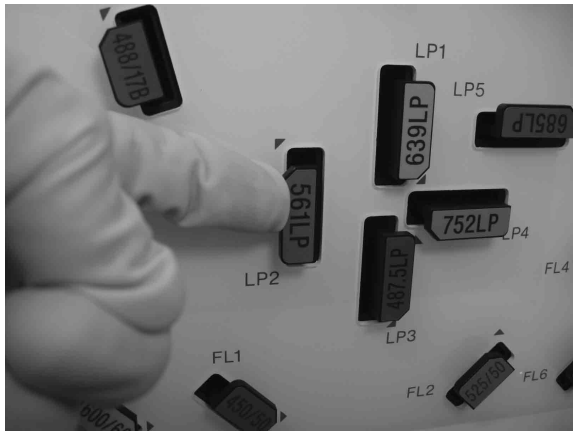
Cleaning and Handling of Optical Filters

The bandpass optical filters and longpass optical filters in the detection module require only light cleaning to remove any dust on the surface of the filters. The filters should be checked and cleaned every 6 to 12 months, or more often if the optical filter pattern is routinely changed.

Note

The optical filters are high-precision components that require the utmost care. If an optical filter is scratched or damaged in any way, it must be replaced.

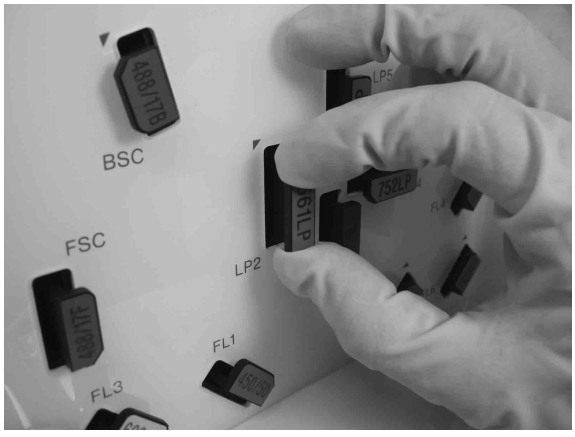
1 Remove an optical filter by placing your finger under the lip of the retaining holder and pulling directly out.



2 Grip the sides of the optical filter and withdraw it from the filter slot.

Note

Do not touch the surfaces of the optical filters.

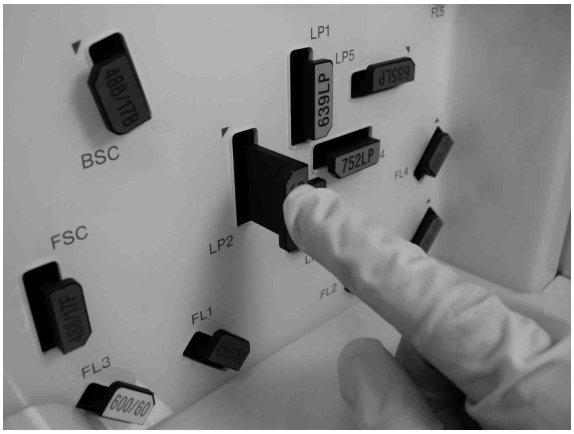


It is recommended that only one optical filter be withdrawn at any one time to ensure that it is returned to the original slot.

3 Remove loose dust or particles using a canned air blower or a bulb puffer hand duster, similar to those used in photography.

Direct the airflow across the surface at an oblique angle. If necessary, gently wipe the surface using lens paper. Use a fresh surface of the cloth for each wipe. Maintain a continuous wiping motion at a constant speed until the cloth passes the edge of the optical filter.

4 After cleaning, insert the optical filter into the slot and push the lip of the optical filter until it clicks into place.



Notes

- Avoid touching or wiping coated or metal mirror surfaces at all times, except when cleaning.
- Avoid handling exposed coatings with bare fingers.
- Always wear gloves, and also wear a face mask, goggles, and other protective clothing as required to prevent oils from your hands contaminating the optical glass, and to prevent solvents and chemicals coming into contact with your skin or eyes.

Disconnecting and Reconnecting the Fluidics Cart

After installation, it may be necessary to disconnect and then reconnect the fluidics cart if you wish to move the fluidics cart and need to re-route the path of the fluidics lines, sheath air line, and cart connection cable between the fluidics cart and the main unit.

Note

If you wish to move the SH800 main unit, contact your Sony distributor. Do not attempt to relocate it yourself. The main unit contains high-precision optical and laser instruments that require alignment after installation that only your Sony distributor can provide.

1 With SH800 Software shut down, turn off the power supply using the MAIN POWER switch on the rear panel of the main unit.

2 Disconnect the three fluidics lines (sheath fluid, waste fluid, and ethanol) and sheath air line from the rear of the fluidics cart.

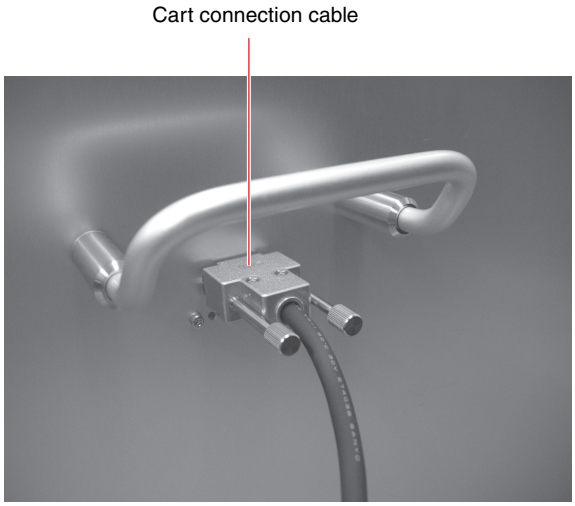
Press the metal release catch to withdraw each connector.

Notes

- Do not touch the surfaces of the connectors that come into contact with fluids. Take care not to spill any fluid.
- Always wear gloves and other protective clothing, mask, and goggles, as required, when handling waste fluid.

3 Disconnect the connection cable from the rear of the fluidics cart.

Unscrew the retaining screws and pull the connector out.



4 Move the fluidics cart or re-route fluidics lines, sheath air line, and cable, as required, then reconnect the lines, air line, and cable.

Insert each connector and push until it locks into place.

5 Turn on the power supply.

Check for any fluid spills, and clean as necessary.

6 Check the operation of the fluid level sensors in the main window in SH800 Software or on the LCD monitor.

Check the electrical cable connection if the status information does not appear.

Cleaning the Internal Sheath Line and DI Water Line using Ethanol

The SH800 internal sheath line and DI water line should be cleaned with ethanol regularly to prevent the growth of bacteria and other agents. In addition, the fluidics system should also be cleaned as required after running samples that contain infectious substances.

Precautions when cleaning with ethanol

- Ensure the laboratory is well ventilated during cleaning with ethanol. Inhaling ethanol vapor can cause irritation of the eyes, skin and respiratory tract, loss of coordination, drowsiness, and in sufficient concentration, unconsciousness.
- Never place a naked flame, embers, or other material that can emit sparks near the main unit or the fluidics cart. Ethanol vapor is highly flammable at normal room temperature.
- Never pour ethanol into a tank other than the ethanol tank, and conversely, never pour other liquids into the ethanol tank.
- Make sure that the cap on the ethanol tank is correctly and securely fastened at all times.
- Make sure that the ethanol line is securely connected to the connectors on the rear panel of the main unit, rear panel of the fluidics cart, and the ethanol tank itself.
- Make sure that the lid on the waste tank is correctly and securely fastened at all times.
- Make sure that the waste fluid line is securely connected to the connectors on the rear panel of the main unit, rear panel of the fluidics cart, and the waste tank itself.

Note

The SH800 is compatible with standard disinfecting agents (ethanol 70%) used for flow cytometers.

1 In SH800 Software, click [Ethanol Cleaning] on the [Cytometer] tab of the ribbon.

Notes

- The ethanol cleaning sequence cannot be aborted once it is started.
- The SH800 hardware and software are automatically shut down after cleaning with ethanol.

You can also run ethanol cleaning in maintenance mode. For details, see “Running Maintenance Mode” (page 158).

2 Click [Start] to begin the cleaning process and follow the on-screen instructions.

3 Refill the DI water tank in the main unit with distilled, deionized water, then click [Next].

Make sure that there is sufficient DI water in the tank. An alarm will be raised if there is insufficient volume of DI water in the DI water tank before cleaning.

See "Refilling the DI Water Tank" (page 149).

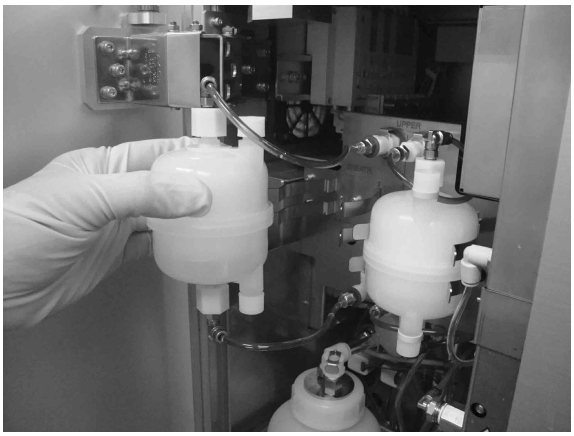
4 Refill the ethanol tank in the fluidics cart with ethanol, then click [Next].

Make sure that there is at least 0.5 liters (18 fl oz.) in the ethanol tank. An alarm will be raised if there is insufficient volume of ethanol in the ethanol tank before cleaning.

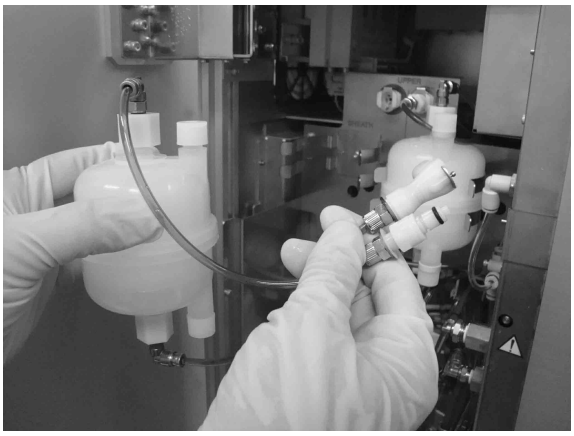
See "Refilling the Ethanol Tank" (page 151).

5 Remove the sheath filter.

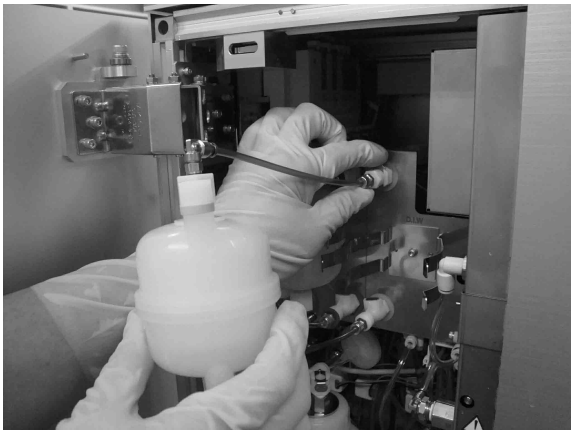
① Remove the sheath filter from the retaining clamp without disconnecting the connectors.

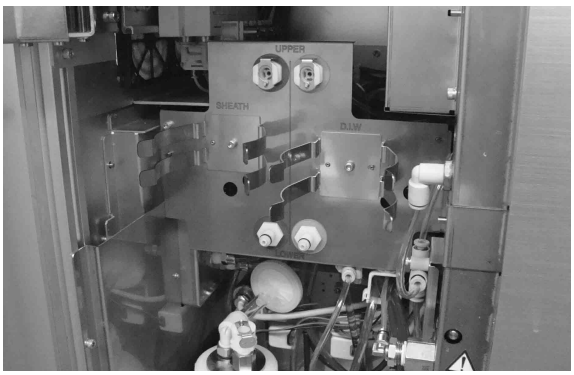


② Disconnect the sheath filter lines from the "UPPER" and "LOWER" connectors.
Press the metal release catch and withdraw each connector. The sheath filter connectors have stop valves that prevent leakage of fluid when disconnected.

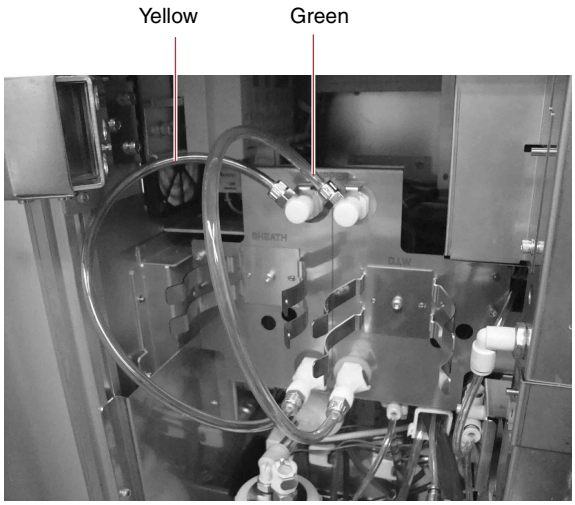


6 Remove the DI water filter in the same way.





7 Connect the sheath filter bypass line (yellow) and DI water filter bypass line (green) to the corresponding “UPPER” and “LOWER” connectors.



8 Hold the QR code on the packaging for the cleaning chip in front of the built-in camera on the PC.

The currently loaded sorting chip, if one is present, is automatically ejected.

9 Insert the cleaning chip in the insertion slot on the top of the chip loader, then click [Next].

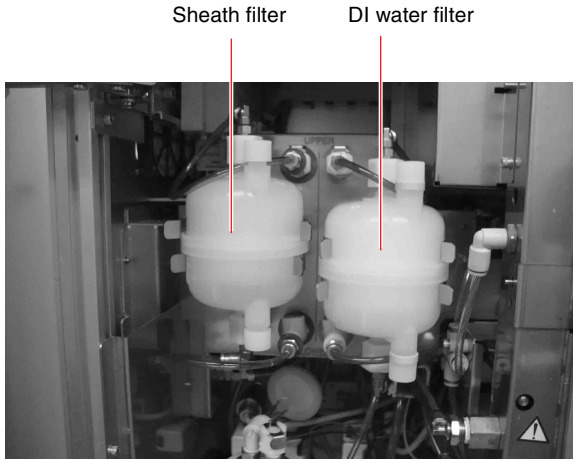
10 Enter the ethanol cleaning time, then click [Start].

The recommended ethanol cleaning time is 12 minutes.
A progress bar is displayed during cleaning.
The sheath fluid line is cleaned with ethanol for the specified duration. When the cleaning time has elapsed, the sheath fluid line is flushed with DI water to remove all traces of ethanol.

11 When cleaning is completed, click [Next].

12 Remove the sheath filter bypass line (yellow) and DI water filter line (green), and reattach the sheath filter and DI water filter.

The DI water filter goes on the right, and the sheath filter on the left. Check that the filters are not connected incorrectly.



13 Click [Next].

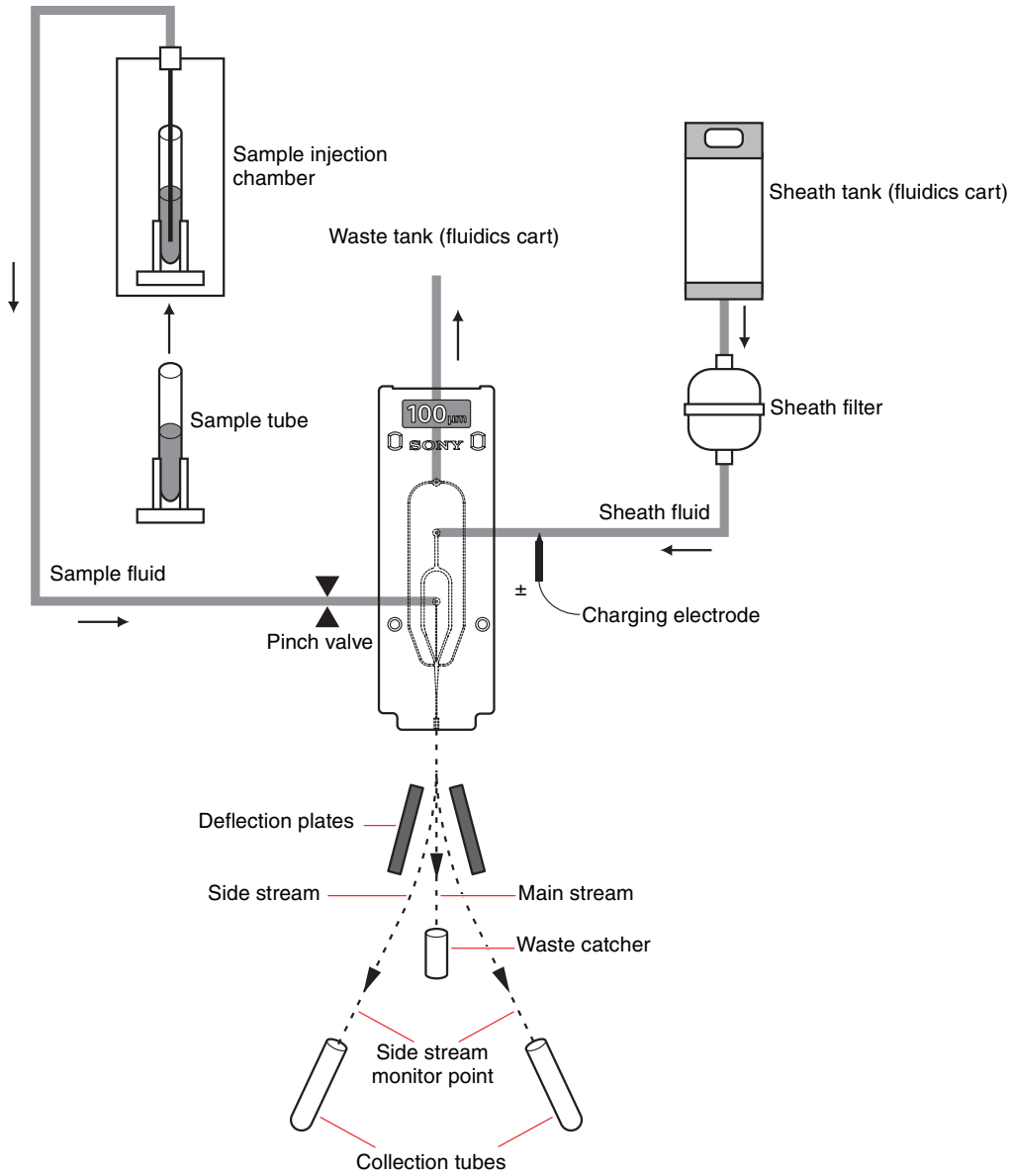
The SH800 hardware and software shut down automatically.
The cleaning chip remains loaded in the chip loader after shutdown.

Operating Principles

Appendix A

This chapter describes some of the basic concepts of flow cytometry and cell sorter operating principles of the SH800.

Fluid Flow



Sample Fluid

Sample preparation is an important part of the testing procedure in flow cytometry. Sample fluid must contain cells in a monodispersed suspension that has been filtered using an appropriate filter mesh to remove aggregates. This is required to maintain a consistent sample concentration. If the sample is not filtered, the sample may clog or adhere to the surfaces of fluidics components, and stable performance cannot be maintained. For more information about sample preparation, refer to reference sources.

Before running samples, check that the sorting chip nozzle size is appropriate for the size of cells in the sample fluid. As a generally accepted rule of thumb, the nozzle size should be approximately five times (min) the diameter of the largest cell in the sample fluid to maintain stable sample fluid flow without clogging the chip.

The standard sorting chip has a 100 µm nozzle, which is suitable for most cell sorting applications. Cells ranging from approximately 1 µm to 20 µm in diameter can be analyzed and sorted.

Note

The size of the sorting chip nozzle affects the droplet size, the gap between droplets, and the consumption of sample fluid and sheath fluid.

Sample fluid can be loaded in 0.5 ml micro tubes, 1.5 ml micro tubes, 5 ml round tubes or 15 ml conical tubes for use with the SH800. Each type of tube fits into a corresponding tube holder for the sample tube. The tube holder all fit into a D-cut slot in the base of the sample loader.

After the sample is loaded and [Start] is clicked in SH800 Software, the sample door closes and the sample tube containing the sample is raised into the injection chamber. The chamber is hermetically sealed and then pressurized using regulated compressed air. The pressure inside the injection chamber forces the sample fluid up through the sample probe and into the sample line connected to the probe at the top of the chamber. Sample fluid flows through the sample line and into the sample fluid inlet port on the front of the sorting chip, via a pinch valve which opens/closes the sample line to control the sample flow.

The sample fluid flow rate is controlled by the [Sample Pressure] setting on the main window in SH800 Software. The sample pressure determines the speed of the sample flow, which indirectly controls the distance between cells in the sample fluid flow as they are injected into the sheath fluid in the sorting chip.

An agitation unit is provided to maintain consistency of the sample fluid during testing. The unit is switched on/off from SH800 Software.

Sheath Fluid

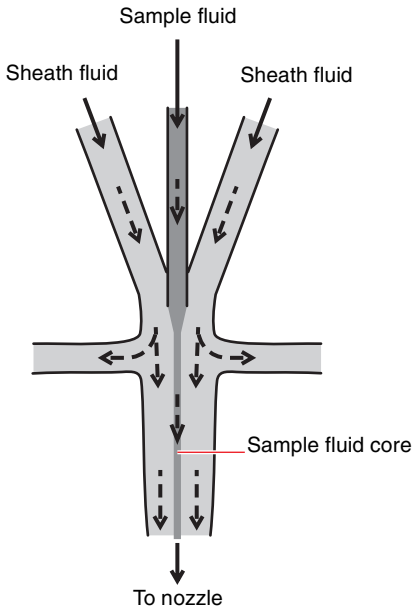
Sheath fluid is supplied from the sheath tank in the fluidics cart. A level sensor in the fluidics cart monitors the level in the tank. The sensor output is displayed on the LCD monitor and on the bottom right of the main window in SH800 Software. A warning is displayed if the level gets close to empty. The tank is pressurized using regulated compressed air, which is used to force the sheath fluid through the fluidics system components at constant pressure, avoiding the pressure fluctuation problems common to pumps.

When [Start] is clicked in SH800 Software to begin sorting, sheath fluid is forced under pressure through the sheath filter and into the sheath fluid inlet port on the front of the sorting chip. A charging electrode mounted near the inlet port is used to electrostatically charge the sheath fluid for sorting.

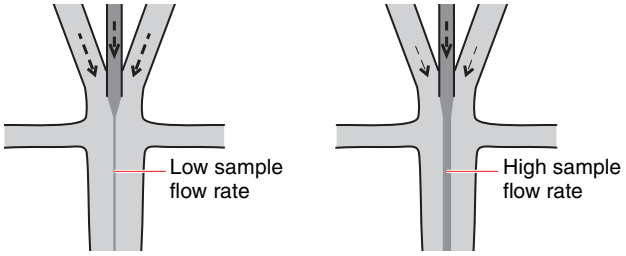
The sheath fluid flow rate is controlled by the [Sheath Pressure] setting in SH800 Software. The sheath pressure determines the speed of the sheath fluid flow through the sorting chip. A low flow rate exposes the cells in the sample fluid to a longer duration of laser excitation energy with larger gaps between cells, providing the highest fluorescence signal. If using fluorochromes with low-efficiency luminescence, decrease the sheath pressure. Increasing the sheath pressure reduces the laser excitation energy received and the intensity of the resulting fluorescence signal, but is effective for increasing the sample throughput rate.

Hydrodynamic Focusing

Sample fluid and sheath fluid are forced into the sorting chip using air pressure. The sample fluid is injected into the center of the sheath fluid stream, hydrodynamically focusing the sample fluid within the sheath fluid, according to the principles of fluid dynamics. The internal micro-channel structure of the sorting chip encases the sample fluid within the sheath fluid to ensure high-sensitivity, stable optical detection characteristics.



The relative difference in pressure between the sheath fluid flow and the sample fluid flow forces the diameter of the sample fluid core to vary. When the sample pressure is lowered, the diameter of the sample fluid core decreases, “focusing” the sample fluid into a narrow core stream. When the sample pressure is raised, the diameter increases with a corresponding increase in the sample event detection rate.

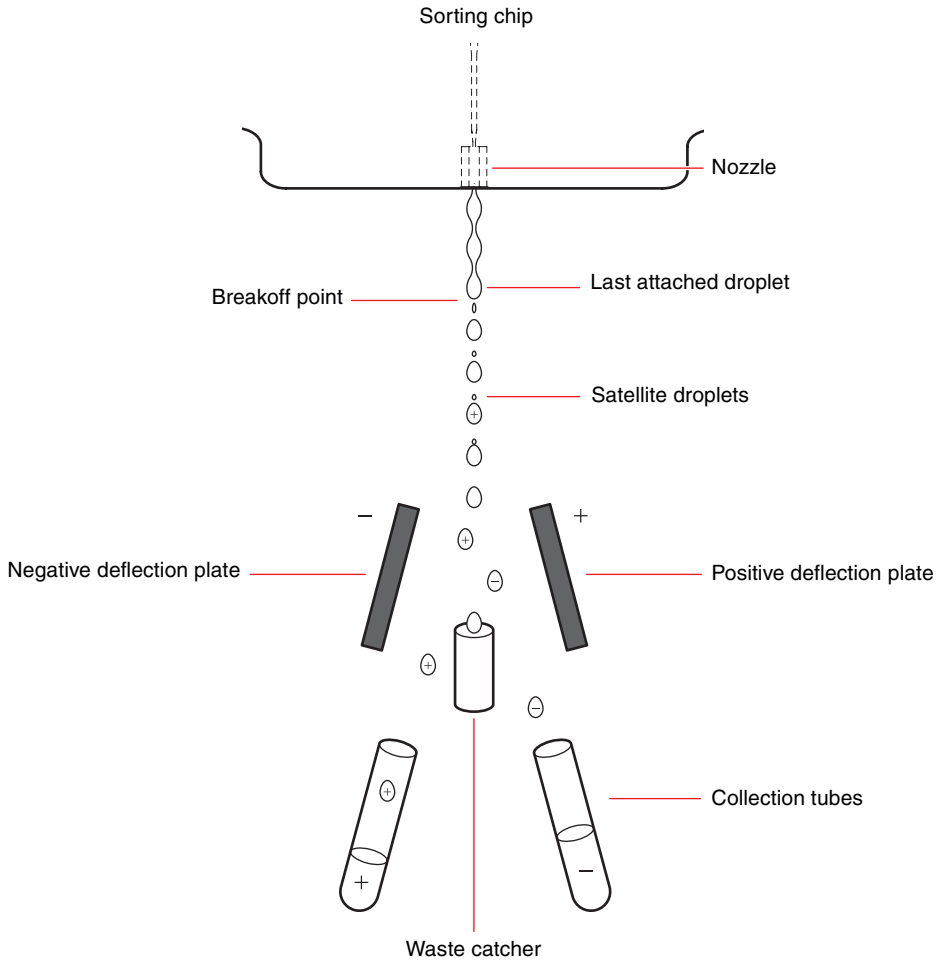


The flow of sample cells encased in sheath fluid passes the optical detection point where the fluid core is interrogated by up to four lasers of different wavelengths.

The highest measurement resolution is obtained when the sample fluid flow is focused into a single-file stream of cells moving past the optical detection point at low sample flow pressure, maximizing the exposure to excitation laser energy that each cell receives.

The diameter of the channel in the sorting chip narrows as it approaches the nozzle at the bottom of the chip, increasing the flow speed while maintaining the relative proportion between the sheath and sample fluid. Steady fluid flow ejected from the nozzle is critical for accurate sorting, hence vibrations of the main unit or the sheath line on the rear panel must be avoided during sorting.

Droplet Sorting



When the sorting chip is inserted in the chip loader, a transducer causes the chip to vibrate at an ultrasonic frequency as fluid flows through the chip. The vibrations cause the fluid flow to be ejected in a jet of uniform droplets from the nozzle at the bottom of the chip.

At a point a short distance beneath the sorting chip, called the breakoff point, the fluid flow breaks into a very regular stream of uniform droplets. The size of the droplets is determined by the nozzle size, frequency of vibration, and the speed of the fluid flow. The amplitude of vibration (drop drive voltage) controls the position of the breakoff point, and the frequency of vibration determines the distance between droplets.

If the last attached droplet satisfies the sorting criteria, the droplet containing the cell receives an electrostatic charge just before the fluid flow reaches the breakoff point from a charging electrode located near the sheath fluid inlet on the sorting chip. The last attached droplet retains the electrical charge after it breaks off from the fluid flow. The electrostatic charge is distributed on the periphery of the droplet and does not affect any sample cells contained within the droplet.

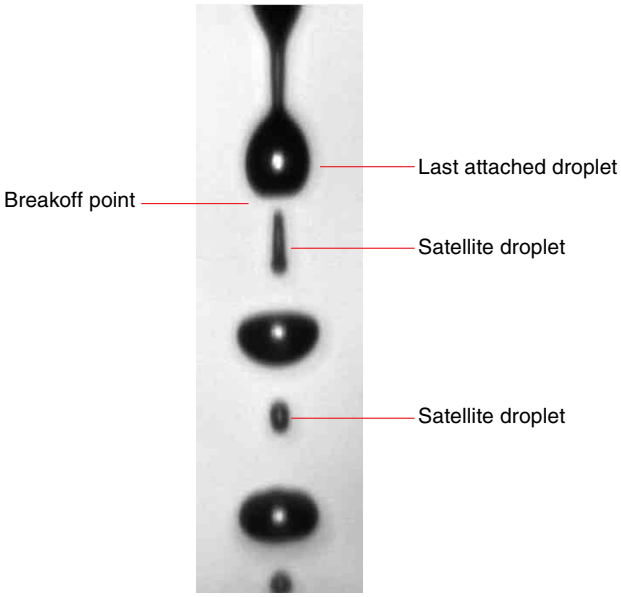
The polarity (positive or negative) of the charge applied to a droplet depends on the cell sorting criteria defined by the characteristics of the cell populations that you want to isolate for sorting using gates specified in SH800 Software. This information is then used to drive the charging pulse for sorting. Unwanted droplets are left uncharged and are collected by the waste catcher.

In an ideal sort, all cells travel pass the optical detection point in single file, and are sorted one cell at a time in separate droplets, with an interval between droplets. In practice, however, cells can appear very close together at the optical detection point, which means they may be found within the same droplet or in adjacent droplets. The sorting mode selection in SH800 Software determines whether to sort or abort droplets whenever these “coincidences” occur.

See “Sorting Mode” (page 93).

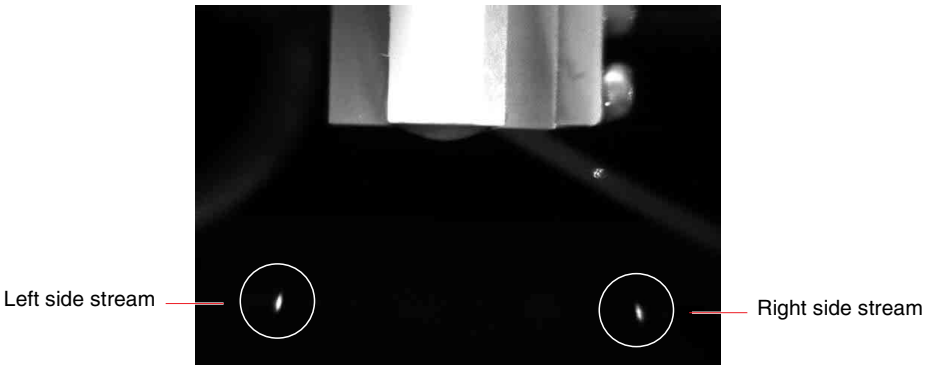
The time taken for droplets to traverse the distance between laser interrogation at the optical detection point and the breakoff point is called the sort delay. The sort delay controls the timing of the electrostatic charging of the target droplet for stable sorting.

The droplet camera monitors the droplet stream before and after the breakoff point. The stroboscopic output image of the droplet camera is displayed in the bottom of the main window in SH800 Software. The output image is also used by control circuits to automatically adjust the amplitude and frequency of vibration of the sorting chip to control the position of the breakoff point and the timing of the electrostatic charging pulse. Manual droplet control is also supported.



High-voltage deflection plates on either side of the droplet stream deflect the charged droplets, using electrostatic attraction and repulsion, to the left and right as the droplets fall between the deflection plates, sorting the droplets into two side streams (two-way sorting). The polarity of the charge on each droplet determines the direction of the stream (left or right). Uncharged droplets (main stream) are not deflected and fall into the waste catcher. The side streams are directed toward the collection tubes on the collection stage.

The side stream monitor detects the position of the side streams. The output image of the side stream camera is displayed in the bottom of the main window in SH800 Software. The output image is also used by control circuits to automatically adjust the angle of deflection of the side streams so that they are directed into the collection tubes. The collection stage supports 15 ml conical tubes and 5 ml round tubes inserted in collection tube holders.



WARNING

The deflection plates are charged at extremely high voltages. Contact with charged deflection plates can result in serious injury or electrical shock. Do not touch the deflection plates while power is connected to the main unit.

The sorting area is sealed during operation to prevent contamination of the air by aerosols generated by falling droplets and to prevent air circulation in the room affecting the accuracy of the droplet sorting. Negative pressure (vacuum suction) helps reduce the risks associated with aerosol generation.

WARNING

Waste fluid may contain biological, chemical, or other agents. Always wear gloves and other protective clothing, mask, and goggles, as required, when handling samples. The handling of waste fluid should be performed in accordance with biological hazard handling safety procedures.

The formation of droplets at the breakoff point and the angular deflection of side streams are calibrated at startup using automatic setup beads.

For details about calibration, see "Automatic Calibration" (page 46).

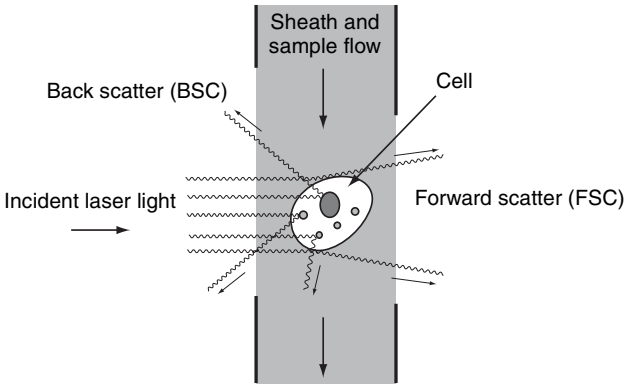
Optical System

This section describes the signals that can be generated using the SH800.

Light and Signal Generation

Light Scatter

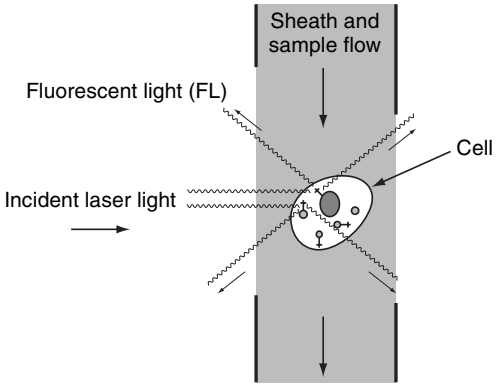
When laser light hits a cell passing the optical detection point in the sorting chip, light is scattered in all directions. Light scattered in the forward direction is called forward scatter (FSC). Light scattered to the sides and back toward the light source is called back scatter (BSC).

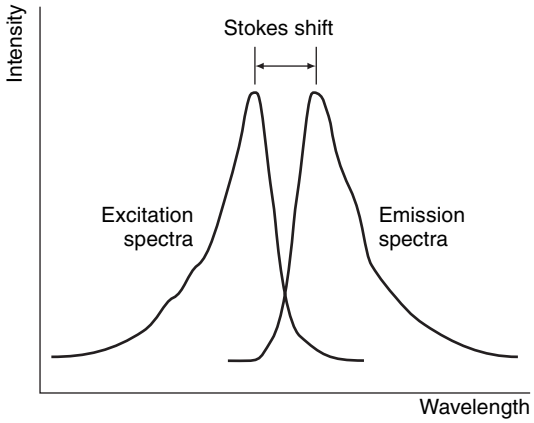


In general terms, forward scatter can provide information about the size of the cell or can indicate the state of living cells. Back scatter can provide information about the internal detail of the cell, indicating complexity, granularity, and irregularities in a cell.

Fluorescence

When laser light hits a cell passing the optical detection point in the sorting chip, the light excitation can cause fluorochromes in molecules or antibodies that have been labeled with fluorescent markers to fluoresce in the sample, producing fluorescent light (FL). Fluorochromes for markers emit high-level fluorescent light when excited by lasers at certain wavelengths and low-level fluorescent light at other wavelengths. The wavelength of the excitation lasers should be selected to match the fluorochromes for the markers.

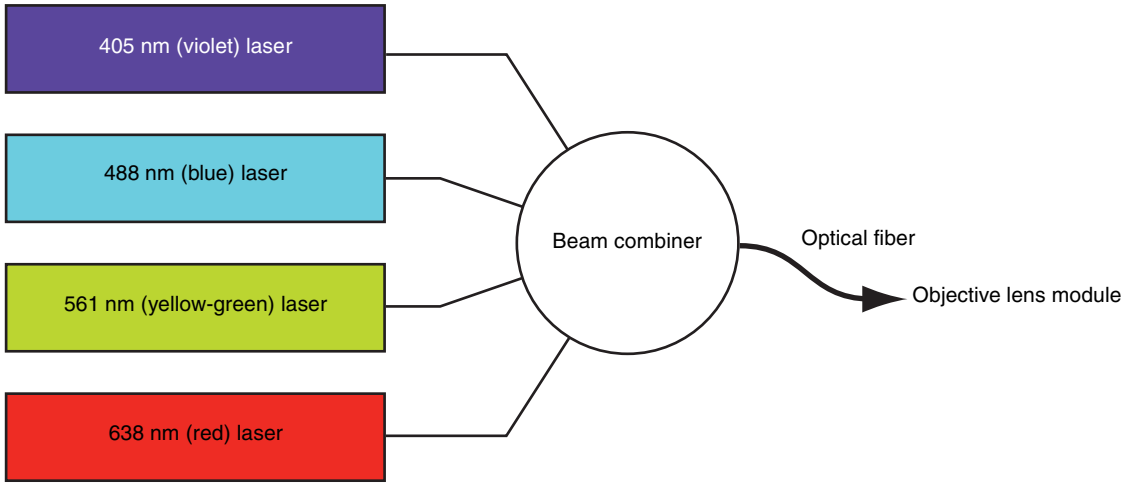




Fluorescence occurs when an electron in a molecule is raised to an excited state as the electron absorbs a photon of the incident excitation light. The electron loses some of this energy before the molecule emits a different photon and the electron returns to its ground state. The emitted photon has less energy than the absorbed photon, so the emitted light has a longer wavelength than the incident light and hence a different color. The difference between the peak excitation wavelength and peak emitted wavelength is called the Stokes shift. Some fluorochromes are available that exhibit a narrow Stokes shift while others exhibit a wide Stokes shift. Multicolor fluorescent light analysis can be used to provide a wealth of structural and other functional information about cells.

Laser Interrogation

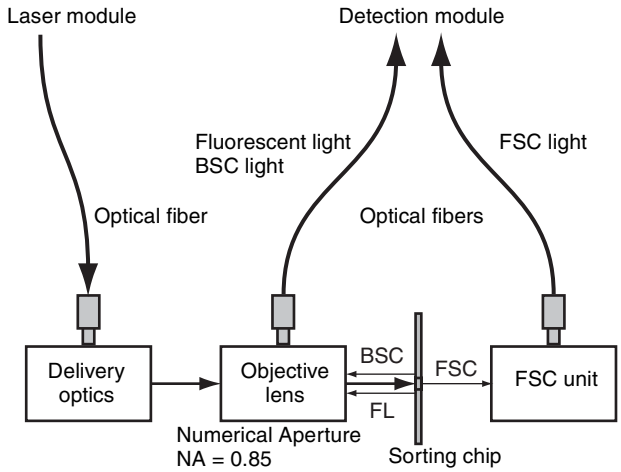
Light source



*** 4-laser models**

The excitation laser module supplies the laser light used to interrogate individual cells as they flow past the optical detection point. The excitation laser beams are combined into a single collinear beam and coupled to a single-mode fiber to the objective lens module. The excitation laser module uses Automatic Power Control (APC) to provide optical measurement accuracy and reproducibility.

Light collection

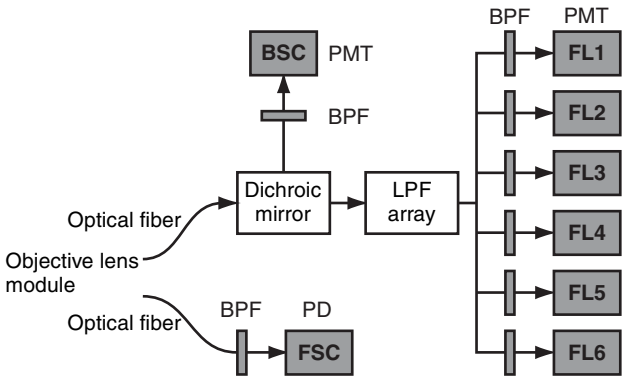


The objective lens module focuses the beam from the laser module onto the optical detection point in the sorting chip.

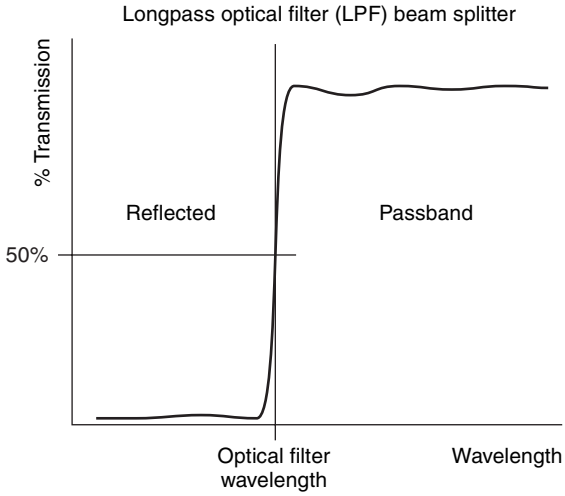
The laser light source from the excitation laser module is focused on the optical detection point in the sorting chip.

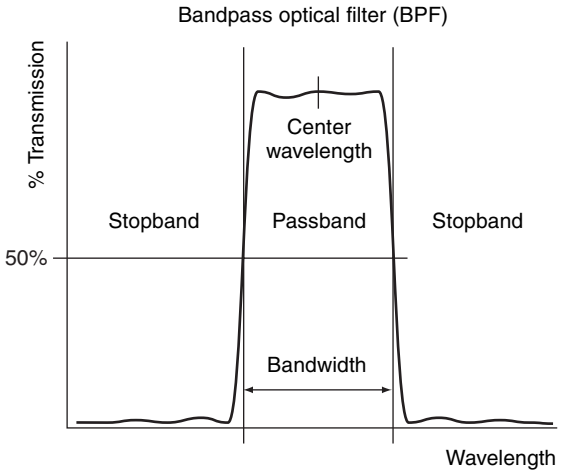
Detection

The detection module takes the light collected from the objective lens module and separates it into eight channels of light, comprising forward scatter light (FSC), back scatter light (BSC), and six channels of fluorescent color light (FL1 to FL6). The output from each channel is received by a detector that converts the light energy into an electrical signal for processing by the acquisition module.



The detection module is comprised by an array of dichroic longpass optical filters (LPF) and bandpass optical filters (BPF). Dichroic longpass optical filters reflect light below a certain wavelength and transmit light above a certain wavelength. Bandpass optical filters transmit light in a narrow band of wavelengths and block light of all other wavelengths. For bandpass optical filters, the first number in the name indicates the center wavelength and the second indicates the bandpass width (e.g. 525/50 is a 525 nm bandpass optical filter with 50 nm bandwidth).





Forward scatter light passes through a collimator and a bandpass optical filter (BPF), and is detected by a photodiode (PD). The bandpass optical filter determines the wavelength of the laser source for which forward scatter is measured.

Note

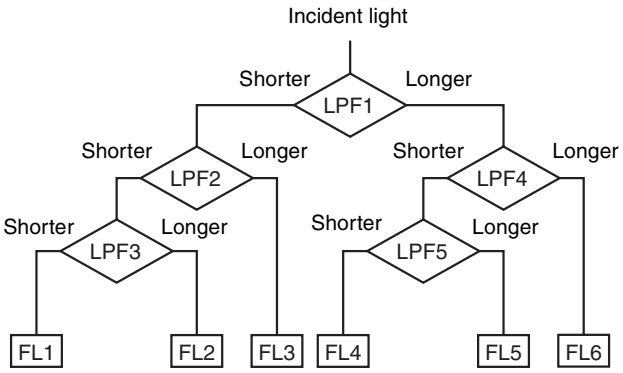
The SH800 is configured by default to measure forward scatter light at a wavelength of 488 nm.

Back scatter light passes through a bandpass optical filter and is detected and amplified by a photomultiplier tube (PMT) detector. The bandpass optical filter determines the wavelength of the laser source for which back scatter is measured.

Note

The SH800 is configured by default to measure back scatter light at a wavelength of 488 nm.

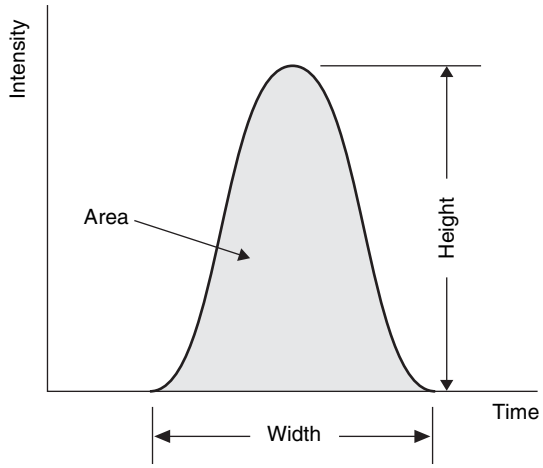
Fluorescent lights passes through an array of longpass optical filters (LPFs), separating the fluorescent light into individual fluorescent channels. Each color then passes through a bandpass optical filter and is detected and amplified by a PMT detector.



The SH800 can detect six channels of fluorescent light simultaneously. The laser configuration and the optical filter pattern determine the fluorescent markers that can be detected.

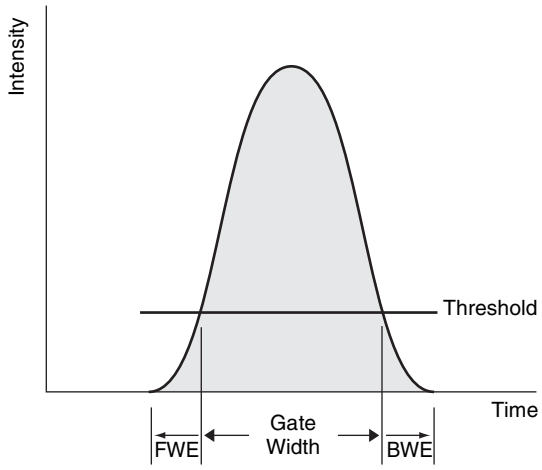
For a list of fluorochromes that can be detected for each model, see "Fluorochrome Detection Matrix" (page 204).

The output from the photodiode and PMT detectors is passed to the acquisition module. The pulse shape that is detected by each detector varies depending on the size and type of cell, and the type of light being collected. The SH800 measures three parameters per pulse (area, height, and width) to provide a wealth of information about cells. The width of the pulse provides an indication of the size of a cell, the height of the pulse provides an indication of the peak fluorescence intensity (brightness) of the cell, and the area provides an indication of the total fluorescent material contained in the cell. The pulse parameters can be selected independently for each channel.



When sorting or analyzing cells, you can set a threshold level for the pulse height for one of the channels (BSC, FSC, FL1 to FL6) to discriminate between real events and background noise. The threshold level for a channel sets the minimum light detector output that is required to record an event. When the trigger is set, data is acquired only for events for which the detector output exceeds the threshold level. Values below the threshold level are ignored. The FSC channel threshold level is the most commonly used trigger, as this channel is less susceptible to the effects of background noise.

The pulse at the threshold level can also be effectively widened to increase the region of the pulse included in the detected signal. This is achieved by adding pulse data that was digitized just before the pulse rises above the threshold value and just after the pulse falls below the threshold value. These two additional components are called forward window extension (FWE) and back window extension (BWE), respectively. Window extensions are used to provide more accurate measurements of the area pulse parameter. When the window extensions are set to zero, only the area above the threshold level is included in the area calculation. By setting window extensions before and after the threshold crossing points, a more accurate determination of the total area under the pulse can be achieved.



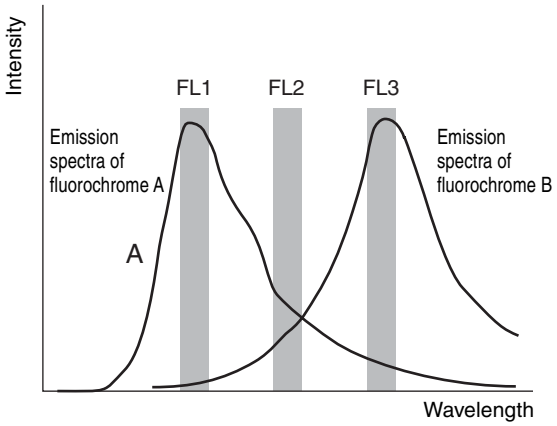
The optimum length of the window extensions depends on the rise height and width of the pulse. Hence, the optimum window extension will vary from pulse to pulse within the same sample, and from experiment to experiment. If the window extensions are too narrow, the measurement for the area pulse parameter will be smaller than its real value. If the window extensions are too wide, then the window extension may begin to overlap with other pulses, degrading the calculation.

When a cell is detected during sorting, it is generally not possible to know with great accuracy if the signal represents the passage of a large single cell, small multiple cells adhering to one another, or other non-cellular material. Accordingly, it is more common to refer to each signal detection not as a cell, but as an event.

Fluorescence Compensation

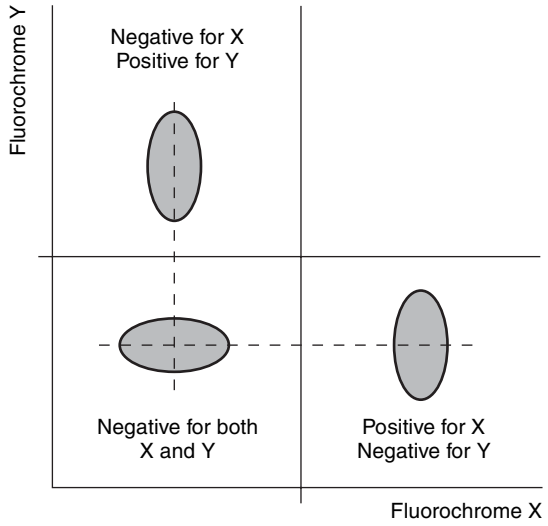
When detecting the emitted fluorescent light for a fluorochrome, components of the emitted light can appear in one or more of the fluorescent light detectors (FL1 to FL6) due to the wide emission spectrum of each fluorochrome. When detecting the emitted light for more than one fluorochrome, components of the spectrum of all fluorochromes can appear in multiple detectors, a

phenomenon called spillover. The problem then becomes one of working out the proportion of the light detected in each detector due to the individual fluorochromes. For example, in the following diagram, the output of detector FL1 is almost totally due to the spectra of fluorochrome A. The output of detector FL3 is predominantly due to the spectra of fluorochrome B, but it does contain a small component (approximately 10%) due to fluorochrome A. However, the output of FL2 contains significant components from the spectra of both fluorochromes A and B. This problem increases considerably as the number of fluorochromes being simultaneously detected increases.



Fluorescence compensation is the process of calculating the components of the detected signals due to each fluorochrome in the sample and subtracting the "unwanted" components from each channel. This converts the channel detector output from signals of fluorescence intensity at a given wavelength to signals of fluorescence intensity due to each fluorochrome.

Compensation adjusts the mean value of fluorescence intensity of populations that are not stained with a particular fluorochrome to the mean value of a negative control sample, which by definition is not stained with any fluorochromes. When compensated, the populations effectively appear aligned into quadrants on 2D plots.



Notes

- The visual representation of the compensated data on a 2D plot will vary depending on the axis type (log, linear, or biexponential).
- The negative and positive populations may or may not appear as diverse populations on 2D plots, depending on the type of cells in the sample and the selected fluorochromes. For example, if samples contain cells that are actively acquiring or losing the antigen with the fluorochrome marker, the two populations may tend to merge into one another.
- SH800 Software also supports manual specification of the fluorescent compensation parameters. See *"Adjusting Fluorescence Compensation Manually" (page 81).*

Miscellaneous

Appendix B

Series Lineup

The SH800 cell sorter is available in six different models equipped with a combination of one, two, three, or four excitation lasers of different wavelengths. The models are indicated by a two-letter suffix (AC to FC).

SH800

Model	No. of lasers	Laser wavelengths (nm)
LE-SH800AC	1	488
LE-SH800BC	2	488, 638
LE-SH800CC	2	488, 405
LE-SH800DC	2	488, 561
LE-SH800EC	3	488, 405, 638
LE-SH800FC	4	488, 405, 638, 561

SH800Z

Model	No. of lasers	Laser wavelengths (nm)
LE-SH800ZA	1	488
LE-SH800ZB	2	488, 638
LE-SH800ZC	2	405, 488
LE-SH800ZD	2	488, 561
LE-SH800ZE	3	405, 488, 638
LE-SH800ZF	4	405, 488, 638, 561
LE-SH800ZG	3	405, 488, 561
LE-SH800ZH	3	488, 638, 561

SH800ZP

Model	No. of lasers	Laser wavelengths (nm)
LE-SH800ZAP	1	488
LE-SH800ZBP	2	488, 638
LE-SH800ZCP	2	405, 488
LE-SH800ZDP	2	488, 561
LE-SH800ZEP	3	405, 488, 638
LE-SH800ZFP	4	405, 488, 638, 561
LE-SH800ZGP	3	405, 488, 561
LE-SH800ZHP	3	488, 638, 561

- 488 nm blue laser (standard)
- 405 nm violet laser
- 561 nm yellow-green laser
- 638 nm red laser

The fluorochromes that can be excited and the resulting emission spectra that can be detected by each laser configuration depend on the wavelength of the lasers and the optical filter pattern. The fluorochrome detection matrix for each laser configuration is given in the *"Fluorochrome Detection Matrix" (page 204)*.

Tip

On multi-laser models, you specify the lasers used for experiments when SH800 Software launches.

Notes

- Do not use the 405 nm laser for extended periods of time. Extended use can damage the sorting chip.
- The use of optical adjustment procedures not specified in this document or installation/removal of optical components may result in hazardous radiation exposure. Do not remove or otherwise modify the excitation laser or detection module.

Optical Filter Patterns

The detection module takes the scattered and fluorescent light collected from cells and separates it into eight channels of light, comprising the forward scatter channel, back scatter channel, and six channels of fluorescent light of various wavelengths.
The SH800 uses a combination of longpass optical filters (in slots labeled LP1 to LP5) and bandpass optical filters (in slots labeled BSC, FSC, and FL1 to FL6) arranged in an optically optimized layout, according to the laser configuration for an experiment.

The SH800 uses two standard optical filter patterns.

Optical filters	Optical filter pattern 1*1	Optical filter pattern 2*2
LP1	639LP	639LP
LP2	600LP	561LP
LP3	561LP	487.5LP
LP4	752LP	752LP
LP5	685LP	685LP
FL1	525/50	450/50
FL2	585/30	525/50
FL3	617/30	600/60
FL4	665/30	665/30
FL5	720/60	720/60
FL6	785/60	785/60
FSC	488/17F	488/17F
BSC	488/17B	488/17B

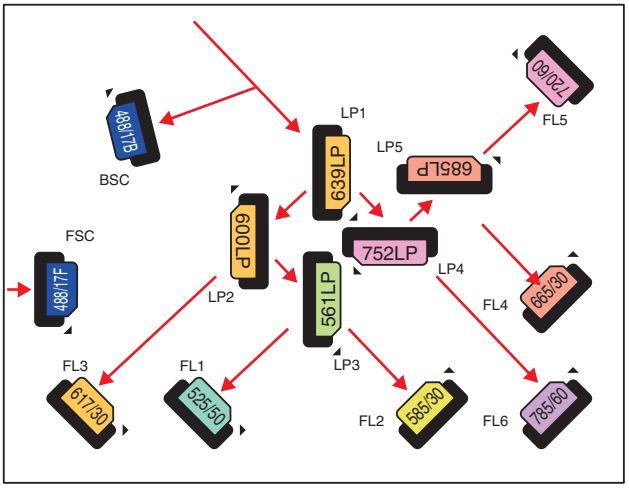
*1. On the following models, dummy filters are installed in the RESERVE slots.
SH800AC, SH800BC, SH800DC, SH800ZA, SH800ZB, SH800ZD, SH800ZH, SH800ZAP, SH800ZBP, SH800ZDP, SH800ZHP

*2. On the following models, filters not used in the current optical filter pattern should be stored in the RESERVE slots. Filter pattern 1 can also be used when the 405 nm laser is turned off to make efficient use of the six fluorescence detection channels.
SH800CC, SH800EC, SH800FC, SH800ZC, SH800ZE, SH800ZF, SH800ZG, SH800ZCP, SH800ZEP, SH800ZFP, SH800ZGP
- Filter pattern 1 can also be used when the 405 nm laser is turned off to make efficient use of the six fluorescence detection channels.
- The shaded portion of the table indicates the filters that are different between optical filter patterns 1 and 2.

Tips

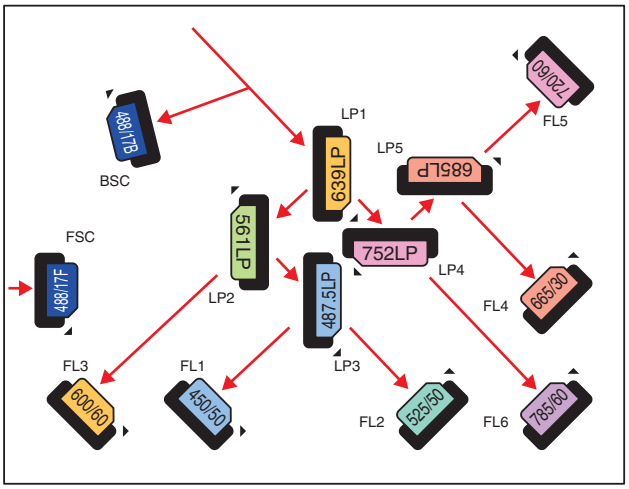
- Optic filter pattern 2 is used only on the following models equipped with a 405 nm laser.
SH800CC, SH800EC, SH800FC, SH800ZC, SH800ZE, SH800ZF, SH800ZG, SH800ZCP, SH800ZEP, SH800ZFP, SH800ZGP
Models other than the above use optic filter pattern 1 only.
- The longpass optical filters are bigger than the bandpass optical filters to prevent the accidental insertion of optical filters in the wrong slots. The LP1 to LP5 longpass optical filters are labeled with the wavelength rating of the optical filter and an "LP" suffix (e.g. "639LP" for 639 nm longpass optical filter). The FL1 to FL6 bandpass optical filters are labeled with the center wavelength of the optical filter and the bandwidth of the optical filter (e.g. "525/50" for 525 nm bandpass optical filter 50 nm bandwidth). The FSC and BSC bandpass optical filter labels have an "F" and "B" suffix, respectively (e.g. "488/17F" for 488 nm bandpass optical filter with 17 nm bandwidth for forward scatter detection).
- Optical filters not used in the current filter pattern can be stored in the RESERVE 1 to 3 slots.

Optical Filter Pattern 1



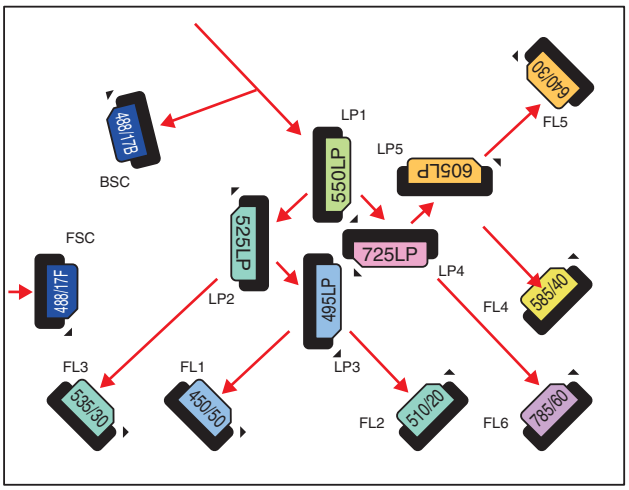
Optical filter label	Installed optical filter
LP1	639LP
LP2	600LP
LP3	561LP
LP4	752LP
LP5	685LP
FL1	525/50
FL2	585/30
FL3	617/30
FL4	665/30
FL5	720/60
FL6	785/60
FSC	488/17F
BSC	488/17B

Optical Filter Pattern 2



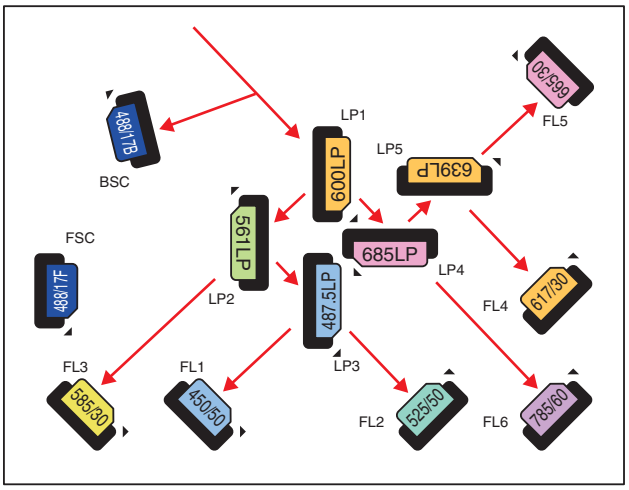
Optical filter label	Installed optical filter
LP1	639LP
LP2	561LP
LP3	487.5LP
LP4	752LP
LP5	685LP
FL1	450/50
FL2	525/50
FL3	600/60
FL4	665/30
FL5	720/60
FL6	785/60
FSC	488/17F
BSC	488/17B

Fluorescent Protein Optical Filter Pattern (Option)



Optical filter label	Installed optical filter
LP1	550LP
LP2	525LP
LP3	495LP
LP4	725LP
LP5	605LP
FL1	450/50
FL2	510/20
FL3	535/30
FL4	585/40
FL5	640/30
FL6	785/60
FSC	488/17F
BSC	488/17B

Filter Pattern for BV/PE/PI



Optical filter label	Installed optical filter
LP1	600LP
LP2	561LP
LP3	487.5LP
LP4	685LP
LP5	639LP
FL1	450/50
FL2	525/50
FL3	585/30
FL4	617/30
FL5	665/30
FL6	785/60
FSC	488/17F
BSC	488/17B

Fluorochrome Detection Matrix

1 laser (488 nm), optical filter pattern 1

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (665/30)
	PE-Cy5	670	FL4 (665/30)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (665/30)
	PE-Cy5.5	695	FL5 (720/60)
	PerCP-Cy5.5	695	FL5 (720/60)
	PerCP-eFluor 710	710	FL5 (720/60)
	PE-Cy7	779	FL6 (785/60)

2 lasers (488 and 638 nm lasers), optical filter pattern 1

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (665/30)
	PE-Cy5	670	FL4 (665/30)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (665/30)
	PE-Cy5.5	695	FL5 (720/60)
	PerCP-Cy5.5	695	FL5 (720/60)
	PerCP-eFluor 710	710	FL5 (720/60)
	PE-Cy7	779	FL6 (785/60)
638 nm laser	APC (Allophycocyanin)	660	FL4 (665/30)
	Cy5	670	FL4 (665/30)
	Alexa Fluor 647	668	FL4 (665/30)
	APC-Cy5.5	695	FL5 (720/60)
	Alexa Fluor 700	719	FL5 (720/60)
	APC-Cy7	779	FL6 (785/60)
	APC-Alexa Fluor 750	775	FL6 (785/60)

2 lasers (405 and 488 nm lasers), optical filter pattern 2

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
405 nm laser	Brilliant Violet 421	421	FL1 (450/50)
	Alexa Fluor 405	421	FL1 (450/50)
	DAPI	455	FL1 (450/50)
	Pacific Blue	455	FL1 (450/50)
	mCFP	475	FL1 (450/50)
	Hoechst 33342	483	FL1 (450/50)
	AmCyan1	491	FL2 (525/50)
	T-Sapphire	511	FL2 (525/50)
	Qdot 525	525	FL2 (525/50)
	Qdot 545	545	FL2 (525/50)
	Pacific Orange	551	FL2 (525/50)
	Brilliant Violet 570	570	FL3 (600/60)
	Qdot 585	588	FL3 (600/60)
	Qdot 605	603	FL3 (600/60)
	Qdot 655	654	FL4 (665/30)
	Qdot 705	705	FL5 (720/60)
	Qdot 800	800	FL6 (785/60)
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (665/30)
	PE-Cy5	670	FL4 (665/30)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (665/30)
	PE-Cy5.5	695	FL5 (720/60)
	PerCP-Cy5.5	695	FL5 (720/60)
	PerCP-eFluor 710	710	FL5 (720/60)
	PE-Cy7	779	FL6 (785/60)

2 lasers (488 and 561 nm lasers), optical filter pattern 1

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (665/30)
	PE-Cy5	670	FL4 (665/30)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (665/30)
	PE-Cy5.5	695	FL5 (720/60)
	PerCP-Cy5.5	695	FL5 (720/60)
	PerCP-eFluor 710	710	FL5 (720/60)
	PE-Cy7	779	FL6 (785/60)
561 nm laser	mOrange	562	FL2 (583/30)
	DsRed-Monomer	586	FL2 (583/30)
	tdTomato	581	FL2 (583/30)
	mCherry	610	FL3 (617/30)
	mPlum	649	FL4 (665/30)

3 lasers (405, 488, and 638 nm lasers), optical filter pattern 2

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
405 nm laser	Brilliant Violet 421	421	FL1 (450/50)
	Alexa Fluor 405	421	FL1 (450/50)
	DAPI	455	FL1 (450/50)
	Pacific Blue	455	FL1 (450/50)
	mCFP	475	FL1 (450/50)
	Hoechst 33342	483	FL1 (450/50)
	AmCyan1	491	FL2 (525/50)
	T-Sapphire	511	FL2 (525/50)
	Qdot 525	525	FL2 (525/50)
	Qdot 545	545	FL2 (525/50)
	Pacific Orange	551	FL2 (525/50)
	Brilliant Violet 570	570	FL3 (600/60)
	Qdot 585	588	FL3 (600/60)
	Qdot 605	603	FL3 (600/60)
	Qdot 655	654	FL4 (665/30)
	Qdot 705	705	FL5 (720/60)
	Qdot 800	800	FL6 (785/60)
488 nm laser	EGFP (Enhanced GFP)	508	FL2 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL2 (525/50)
	FITC	518	FL2 (525/50)
	Alexa Fluor 488	519	FL2 (525/50)
	EYFP (Enhanced YFP)	527	FL2 (525/50)
	mCitrine	529	FL2 (525/50)
	PE (R-Phycoerythrin)	576	FL3 (600/60)
	PE-Texas Red	615	FL3 (600/60)
	PI (Propidium Iodide)	617	FL3 (600/60)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (665/30)
	PE-Cy5	670	FL4 (665/30)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (665/30)
	PE-Cy5.5	695	FL5 (720/60)
	PerCP-Cy5.5	695	FL5 (720/60)
	PerCP-eFluor 710	710	FL5 (720/60)
	PE-Cy7	779	FL6 (785/60)
638 nm laser	APC (Allophycocyanin)	660	FL4 (665/30)
	Cy5	670	FL4 (665/30)
	Alexa Fluor 647	668	FL4 (665/30)
	APC-Cy5.5	695	FL5 (720/60)
	Alexa Fluor 700	719	FL5 (720/60)
	APC-Cy7	779	FL6 (785/60)
	APC-Alexa Fluor 750	775	FL6 (785/60)

3 lasers (405, 488, and 561 nm lasers), optical filter pattern 2

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
405 nm laser	Brilliant Violet 421	421	FL1 (450/50)
	Alexa Fluor 405	421	FL1 (450/50)
	DAPI	455	FL1 (450/50)
	Pacific Blue	455	FL1 (450/50)
	mCFP	475	FL1 (450/50)
	Hoechst 33342	483	FL1 (450/50)
	AmCyan1	491	FL2 (525/50)
	Brilliant Violet 510	510	FL2 (525/50)
	T-Sapphire	511	FL2 (525/50)
	Qdot 525	525	FL2 (525/50)
	Qdot 545	545	FL2 (525/50)
	Pacific Orange	551	FL2 (525/50)
	Brilliant Violet 570	570	FL3 (600/60)
	Qdot 585	588	FL3 (600/60)
	Qdot 605	603	FL3 (600/60)
	Brilliant Violet 605	605	FL3 (600/60)
	Brilliant Violet 650	650	FL4 (665/30)
	Qdot 655	654	FL4 (665/30)
	Qdot 705	705	FL5 (720/60)
	Brilliant Violet 711	711	FL5 (720/60)
	Brilliant Violet 785	785	FL6 (785/60)
	Qdot 800	800	FL6 (785/60)
488 nm laser	EGFP (Enhanced GFP)	508	FL2 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL2 (525/50)
	FITC	518	FL2 (525/50)
	Alexa Fluor 488	519	FL2 (525/50)
	SYBR Green ?	522	FL2 (525/50)
	EYFP (Enhanced YFP)	527	FL2 (525/50)
	Vinus	528	FL2 (525/50)
	mCitrine	529	FL2 (525/50)
	PE (R-Phycoerythrin)	576	FL3 (600/60)
	PE-Texas Red	615	FL3 (600/60)
	PI (Propidium Iodide)	617	FL3 (600/60)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (665/30)
	PE-Cy5	670	FL4 (665/30)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (665/30)
	PE-Cy5.5	695	FL5 (720/60)
	PerCP-Cy5.5	695	FL5 (720/60)
	PerCP-eFluor 710	710	FL5 (720/60)
	PE-Cy7	779	FL6 (785/60)
561 nm laser	mOrange	562	FL3 (600/60)
	DsRed-Monomer	586	FL3 (600/60)
	tdTomato	581	FL3 (600/60)
	mCherry	610	FL3 (600/60)
	mPlum	649	FL4 (655/30)

3 lasers (405, 561, and 638 nm lasers), optical filter pattern 1

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
488 nm laser	EGFP (Enhanced GFP)	508	FL1 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL1 (525/50)
	FITC	518	FL1 (525/50)
	Alexa Fluor 488	519	FL1 (525/50)
	SYBR Green ?	522	FL1 (525/50)
	EYFP (Enhanced YFP)	527	FL1 (525/50)
	Venus	528	FL1 (525/50)
	mCitrine	529	FL1 (525/50)
	PE (R-Phycoerythrin)	576	FL2 (585/30)
	PE-Texas Red	615	FL3 (617/30)
	PI (Propidium Iodide)	617	FL3 (617/30)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (655/30)
	PE-Cy5	670	FL4 (655/30)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (655/30)
	PE-Cy5.5	695	FL5 (720/60)
	PerCP-Cy5.5	695	FL5 (720/60)
	PerCP-eFluor 710	710	FL5 (720/60)
	PE-Cy7	779	FL6 (785/60)
561 nm laser	mOrange	562	FL2 (585/30)
	DsRed-Monomer	586	FL2 (585/30)
	tdTomato	581	FL2 (585/30)
	mCherry	610	FL3 (617/30)
	mPlum	649	FL4 (655/30)
638 nm laser	APC (Allophycocyanin)	660	FL4 (655/30)
	Cy5	670	FL4 (655/30)
	Alexa Fluor 647	668	FL4 (655/30)
	APC-Cy5.5	695	FL5 (720/60)
	Alexa Fluor 700	719	FL5 (720/60)
	APC-Cy7	779	FL6 (785/60)
	APC-Alexa Fluor 750	775	FL6 (785/60)

4 lasers (405, 488, 561, and 638 nm lasers), optical filter pattern 2

Excitation laser	Fluorochrome		Channel (optical filter)
	Name	Peak emission wavelength (nm)	
405 nm laser	Brilliant Violet 421	421	FL1 (450/50)
	Alexa Fluor 405	421	FL1 (450/50)
	DAPI	455	FL1 (450/50)
	Pacific Blue	455	FL1 (450/50)
	mCFP	475	FL1 (450/50)
	Hoechst 33342	483	FL1 (450/50)
	AmCyan1	491	FL2 (525/50)
	T-Sapphire	511	FL2 (525/50)
	Qdot 525	525	FL2 (525/50)
	Qdot 545	545	FL2 (525/50)
	Pacific Orange	551	FL2 (525/50)
	Brilliant Violet 570	570	FL3 (600/60)
	Qdot 585	588	FL3 (600/60)
	Qdot 605	603	FL3 (600/60)
	Qdot 655	654	FL4 (665/30)
	Qdot 705	705	FL5 (720/60)
	Qdot 800	800	FL6 (785/60)
488 nm laser	EGFP (Enhanced GFP)	508	FL2 (525/50)
	CFSE (Carboxyfluorescein Succinimidyl ester)	517	FL2 (525/50)
	FITC	518	FL2 (525/50)
	Alexa Fluor 488	519	FL2 (525/50)
	EYFP (Enhanced YFP)	527	FL2 (525/50)
	mCitrine	529	FL2 (525/50)
	PE (R-Phycoerythrin)	576	FL3 (600/60)
	PE-Texas Red	615	FL3 (600/60)
	PI (Propidium Iodide)	617	FL3 (600/60)
	7-AAD (7-Aminoactinomycin D)	647	FL4 (665/30)
	PE-Cy5	670	FL4 (665/30)
	PerCP (Peridinin chlorophyll protein)	675	FL4 (665/30)
	PE-Cy5.5	695	FL5 (720/60)
	PerCP-Cy5.5	695	FL5 (720/60)
	PerCP-eFluor 710	710	FL5 (720/60)
	PE-Cy7	779	FL6 (785/60)
561 nm laser	mOrange	562	FL3 (600/60)
	DsRed-Monomer	586	FL3 (600/60)
	tdTomato	581	FL3 (600/60)
	mCherry	610	FL3 (600/60)
	mPlum	649	FL4 (665/30)
638 nm laser	APC (Allophycocyanin)	660	FL4 (665/30)
	Cy5	666	FL4 (665/30)
	Alexa Fluor 647	668	FL4 (665/30)
	APC-Cy5.5	695	FL5 (720/60)
	Alexa Fluor 700	719	FL5 (720/60)
	APC-Cy7	779	FL6 (785/60)
	APC-Alexa Fluor 750	775	FL6 (785/60)

Relationship between Sample Pressure and Sample Flow/Event Rate

100 µm sorting chip

[Sample Pressure]		1	2	3	4	5	6	7	8	9	10
Flow rate (µl/min)		6	11	16	21	27	37	47	58	68	89
Event rate (eps)	Sample concentration (1e6/ml)	60	160	260	350	450	650	840	1,000	1,200	1,600
	Sample concentration (1e7/ml)	600	1,300	2,100	2,800	3,600	5,100	6,600	8,100	9,600	12,600

130 µm sorting chip

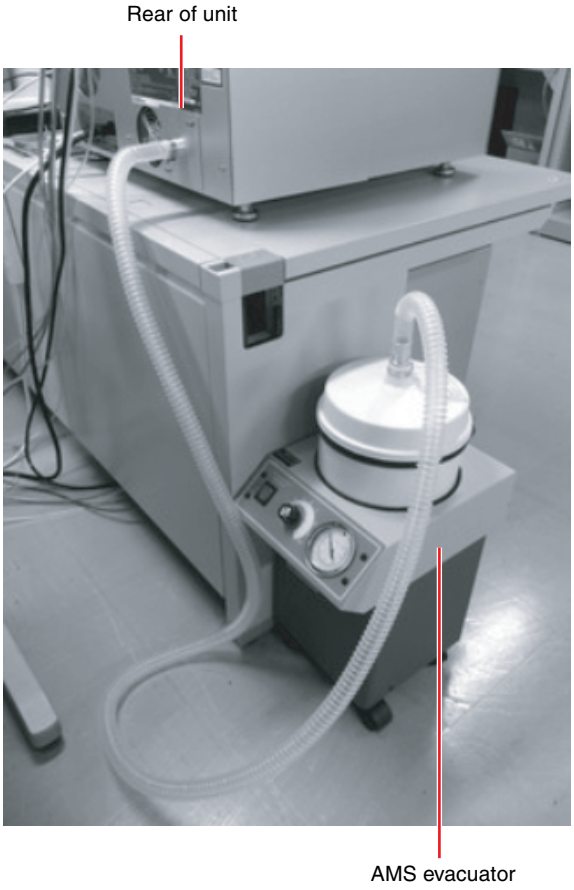
[Sample Pressure]		1	2	3	4	5	6	7	8	9	10
Flow rate (µl/min)		1	3	7	10	16	22	28	34	40	50
Event rate (eps)	Sample concentration (1e6/ml)	10	30	100	150	250	400	500	600	700	900
	Sample concentration (1e7/ml)	100	600	1,200	2,000	2,800	3,600	4,500	5,400	6,500	8,000

Connecting an AMS Evacuator (SH800Z and SH800ZP)

You can connect a S.A.F.E. System (evacuator) from Edge Systems LLC using a specialized adapter (AMS evacuator connection kit).

Note

Compliance with US NIH (National Institutes of Health) biosafety policies is not guaranteed.



For details on operating the evacuator and replacing filters, refer to the operating instructions for the evacuator.

AMS evacuator connection kit components

- HEPA filter bracket (1)
- AMS tube joint (1)
- Screws (2)

Note

AMS evacuator installation should be performed by service personnel. However, you can attach and remove of the vacuum tubing by yourself as follows.

Connecting the vacuum tubing

1 Verify that the unit and the evacuator are turned off.

2 Attach one end of the vacuum tubing to the HEPA filter bracket.

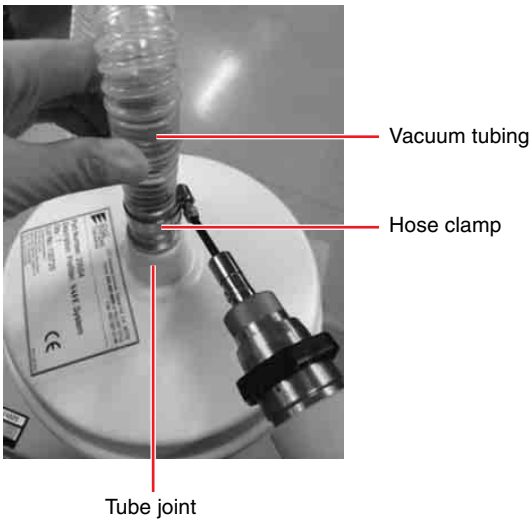
① Place a hose clamp on the vacuum tubing, and attach the tubing to the HEPA filter bracket.

② Secure the hose clamp using a Phillips screwdriver.

3 Attach the other end of the vacuum tubing to the AMS evacuator.

① Place a hose clamp on the vacuum tubing, and attach the tubing to the AMS evacuator's tube joint.

② Secure the hose clamp using a Phillips screwdriver.



4 Turn on the evacuator at the evacuation level specified during installation.

5 Turn on the unit.

Notes

- The evacuator must be turned on when performing automatic calibration on the unit. If the evacuator was not turned on, turn on the evacuator before performing automatic calibration again.
- Position the vacuum tubing so that it is not in contact with the unit, fluidics cart, and sheath line.

Disconnecting the vacuum tubing

Perform the procedure for connection in reverse.

Troubleshooting

This section lists common problems that may occur and the actions to take to resolve each problem. Check these items before calling for service. If the problem still persists after carrying out the recommended actions, contact your Sony distributor.

Problem	Possible Cause/Recommended Solution
The event rate does not rise during data acquisition, sorting, or adjustment.	The sample line may be clogged. Click [Probe Wash] in the [Cleaning] group on the [Cytometer] tab of the ribbon to clean the sample line. If the problem persists, change the sample line *(page 172)*.
Measurement values cannot be acquired.	The sorting chip may be clogged or contain trapped air. Click [Chip] in the [De-bubble] group on the [Cytometer] tab of the ribbon several times as required to clear the chip. If the problem persists, the objective module lens may require cleaning. Contact your Sony distributor for service.
Droplet image in droplet viewer shakes up and down, or flows in a curved path.	The sorting chip may be clogged or contain trapped air. Click [Chip] in the [De-bubble] group on the [Cytometer] tab of the ribbon several times as required to clear the chip. If the problem persists, replace the sorting chip. The fluid consistency of the sample may be causing fluctuations in the sample flow. Click [Agitate] on the [Cytometer] tab of the ribbon to turn on sample agitation. Check that there is no air leaking from the sheath tank. If air is leaking, reattach the lid until it is correctly sealed. Check that there are no air bubbles in the sheath filter. If the problem persists, there may be air bubbles trapped in the sheath filter. Remove the air bubbles from the sheath filter. See *"Releasing Air in the Sheath Filter and Sorting Chip" (page 181)*. If the problem persists, there may be salt deposits in the flow channels. Log off and restart in maintenance mode (*"Running Maintenance Mode" (page 158)*). The sorting chip is ejected automatically. Check that the surface of the chip is not dirty or smudged, then perform chip realignment. If the sorting chip is dirty or smudged, replace the chip. If the problem persists, contact your Sony distributor for service.
Shape of droplets is irregular.	The sorting chip may contain trapped air. Check that there is no air trapped in the sheath filter. Click [Chip] in the [De-bubble] group on the [Cytometer] tab of the ribbon several times as required to clear the chip. Check that there are no air bubbles in the sheath filter. If the problem persists, there may be air bubbles trapped in the sheath filter. Remove the air bubbles from the sheath filter. See *"Releasing Air in the Sheath Filter and Sorting Chip" (page 181)*. If the problem persists, there may be salt deposits in the flow channels. Log off and restart in maintenance mode (*"Running Maintenance Mode" (page 158)*). The sorting chip is ejected automatically. Check that the surface of the chip is not dirty or smudged, then perform chip realignment. If the sorting chip is dirty or smudged, replace the chip. The sorting parameters may be set incorrectly. The sorting parameters affect the droplet shape, breakoff point, and electric charge. Increase the amplitude or decrease the frequency to make droplets larger. Decrease the amplitude or increase the frequency to make droplets smaller.
Sheath fluid is not flowing.	Check that the connectors for all fluidics lines in the fluidics cart and main unit are connected securely and that the lid of the sheath tank is closed tightly. Check that the sheath tank lid has a black rubber seal attached. Check that there are no air bubbles in the sheath filter. Check the pressure of the compressed air supply to the main unit.
Electrical discharge between deflection plates causes sorting errors.	Discharge may occur due to fluid droplets adhering to the surface of the plates. Turn off the power, open the sorting area door, and wipe the deflection plates dry. If residue remains on the plates, remove and clean the plates.
PMT output signal does not change after increasing the gain.	Increasing the gain too much can cause output saturation. In this case, reduce the gain and readjust settings to find the optimum operating point.
Sorting chip does not load correctly.	Log off and restart in maintenance mode (*"Running Maintenance Mode" (page 158)*). If the problem persists, replace the sorting chip. If the problem persists, contact your Sony distributor for service.
Chip does not eject.	Use the manual load/unload thumbwheel on the chip loader to remove the chip.

Problem	Possible Cause/Recommended Solution
Sorting chip clogs after changing the sheath filter.	There may be some small particles in the replacement filter that have not been removed. Run the sheath filter de-bubble function at least three times.
Sample tube hits the sample probe.	Use only standard tubes and fully insert the tube in the sample tube holder when loading a sample. An ill-fitting or non-standard tube can damage the sample probe.
Fluid is leaking from the sample probe.	Check that the sample line is fully inserted in the pinch valve.
Fluid is leaking from the top in the sorting area.	The sample fluid connector may not be securely fastened. Securely fasten the sample fluid connector.
Fluidics component (pump, fluid connectors, etc.) is leaking fluid.	The seals may have deteriorated due to age, causing ethanol or waste fluid to leak. Clean the area, and contact your Sony distributor for service. **Note** Always wear gloves and other protective clothing, mask, and goggles, as required, when cleaning spills and leakages.
Droplet camera image is erratic.	The droplet camera may require cleaning. Contact your Sony distributor for service.
Droplets are not being formed or the droplet camera image is not displayed.	The droplet camera may be defective. Contact your Sony distributor for service.
Data is not acquired in a given fluorescence detection channel or condition less than favorable.	The optical filters may not be inserted in the correct filter pattern or may require cleaning. Check that the filters are clean and inserted in the correct slots.
Gain settings appear to have deviated during measurement.	The optical filters may have been subjected to static electricity discharge. Pause or stop data acquisition, and then resume data acquisition to restore normal gain settings.
No output from the lasers (laser temperature error).	If the ambient operating temperature exceeds the maximum operating temperature of 35 °C (95 °F), the lasers shutdown automatically and an error message is displayed. To clear the error, shut down the main unit and wait for the ambient temperature to cool sufficiently before restarting the main unit. If the error persists even after allowing the ambient temperature to cool below 35 °C (95 °F), contact your Sony distributor for service. If the ambient temperature drops below the minimum operating temperature of 10 °C (50 °F), the lasers shut down automatically and an error message is displayed. To clear the error, shut down the main unit and wait for the ambient temperature to rise above 10 °C (50 °F) before restarting the main unit. If the error persists even after the temperature has risen above 10 °C (50 °F), contact your Sony distributor for service.
Expected data is not acquired using reference beads.	Check the beads lot and dilution/concentration.
Waste fluid is not being collected in the waste tank.	Waste fluid may be leaking in the fluidics cart due to an unsecured waste line connector on the waste tank. Make sure the waste line connector is securely connected on the waste tank. Replace the waste tank air filter if more than one month has elapsed since the air filter was last replaced. If the problem persists, contact your Sony distributor for service.
Communication error between the SH800 and the host computer.	Shutdown and restart both the SH800 main unit and the host computer.
POWER/STANDBY button indicator is flashing.	If the doors can be opened and closed, remove the sample tube and collection tubes before performing the following procedure. **1** Press and hold the POWER/STANDBY button until the indicator stops flashing. **2** Press the POWER/STANDBY button again to start the main unit. If the POWER/STANDBY button indicator does not flash, continue normal operation. **3** If the POWER/STANDBY button indicator starts flashing again, press and hold the POWER/STANDBY button until the indicator stops flashing, then turn off the main power switch on the rear panel. **4** Contact your Sony distributor for service.

Problem	Possible Cause/Recommended Solution
An error occurs in automatic calibration or automatic calibration stops and does not proceed.	• There may be air bubbles trapped in the sheath filter. Remove the air bubbles from the sheath filter. See *"Releasing Air in the Sheath Filter and Sorting Chip" (page 181)*. • If an error occurs while adjusting the side streams, the sensor windows may be dirty, preventing normal side stream detection. Clean the windows using a soft, lint-free cloth lightly moistened with water. Clean the black sensor windows on the left, right, and back. • The temperature within the unit may have changed. With the main unit up and running, leave standing until the room temperature stabilizes, then run automatic calibration again. • If the problem persists, there may be salt deposits in the flow channels. Log off and run the unit in maintenance mode (*"Running Maintenance Mode" (page 158)*). • If the problem persists, change the sorting chip. • If the problem persists, contact your Sony distributor for service.
Side streams are not visible during automatic calibration.	The transparent protective covers in the sorting area may be charged with static electricity. Clean the covers using a soft, lint-free cloth moistened with water. For details, see *"General Cleaning" (page 153)*.
The output pressure of the air compressor drops.	Connect the power cord for the air compressor directly to a power outlet. Do not connect using an extension cord.
The waste tank is bulging.	The waste tank air filter may be blocked. Replace the waste tank air filter. See *"Changing Tank Air Filters" (page 165)*.
Water splashes occur when cleaning the sample probe.	De-bubble the DI water filter. For details, see *"Changing the DI Water Filter" (page 177)*.
The instrument will not operate after adding a printer in [Control Panel] on the host computer.	Adding a printer in [Control Panel] while the SH800 main unit and host computer are connected may cause the SH800 main unit to stop functioning.Press and hold the POWER/STANDBY button on the front panel to turn off the power, then turn the power on again and restart SH800 Software. To add a printer, first turn off the SH800 power supply or disconnect the network cable before adding the printer.
The main window in SH800 Software does not update (e.g. the event rate does not update).	If the main window display does not update, click the SH800 icon in the task bar to minimize the main window. Then, click the SH800 icon again to restore the window. If pull-down menus still do not open in the main window, open the [Detector & Threshold Settings] dialog *(page 79)* and operate a [Threshold] pull-down menu, for example.
Button labels displayed for dialogs on the main window are transparent.	If the dialog display is corrupted, press the ESC key to close the dialog (clicking the [X] icon in the top right corner of the dialog has no affect). Then, reopen the dialog from the main window. If the problem persists, click the SH800 icon in the task bar to minimize the main window and then click it again to restore the window.

Error Messages

This section lists the error messages that may be displayed in SH800 Software. When an error occurs, check these items for the actions to take to resolve each error. If the problem still persists after carrying out the recommended actions, make a note of the error message and then contact your Sony distributor.

GUI Error Message	Recommended Solution
Unload Chip Error. Discard and load a new chip. If the error reoccurs, please contact your service representative.	Shut down the cytometer and turn off the compressed air supply. Pull the sample line connector out slightly from the chip loader, and turn the Emergency thumbwheel to eject the chip.
Load Chip Error. Error on loading mechanism. Please reinsert the chip. If the error reoccurs, please contact your service representative.	Insert a new chip and try to load the chip again. If the problem persists, contact your Sony distributor for service.
Host Communication Error. **1** Shutdown the cytometer. **2** Make sure the cable is connected correctly and firmly and restart the cytometer and software. If the error reoccurs, please contact your service representative.	Check the connection between the cytometer and the host computer and reboot the SH800 Software. If the problem persists, contact your Sony distributor for service.
Unload Sample tube Error. If the error reoccurs, please contact your service representative.	**1** Check that the compressed air supply is supplying rated pressure. **2** Shut down the cytometer, and turn off the compressed air supply. **3** Clean or replace the sample loader O-ring. If the problem persists, contact your Sony distributor for service.
Unload Sample tube Error. Failed to reduce the sample pressure. If the error reoccurs, please contact your service representative.	
Unload Sample tube Error. Failed to down the sample loader. If the error reoccurs, please contact your service representative.	
Unload Sample tube Error. Failed to move the support guide. If the error reoccurs, please contact your service representative.	
Unload Sample tube Error. Failed to move the sample stage. If the error reoccurs, please contact your service representative.	
Load Sample tube Error. Please reload the tube. If the error reoccurs, please contact your service representative.	
Load Sample tube Error. Failed to move the waste tray. Please reload the tube. If the error reoccurs, please contact your service representative.	
Load Sample tube Error. Failed to lift up the sample loader. Please reload the tube. If the error reoccurs, please contact your service representative.	

GUI Error Message	Recommended Solution
Load Sample tube Error. Failed to move the support guide. Please reload the tube. If the error reoccurs, please contact your service representative.	**1** Check that the compressed air supply is supplying rated pressure. **2** Shut down the cytometer, and turn off the compressed air supply. **3** Clean or replace the sample loader O-ring. If the problem persists, contact your Sony distributor for service.
Load Sample tube Error. Failed to move the sample stage. Please reload the tube. If the error reoccurs, please contact your service representative.	
Sheath Fluid Tank is empty. Refill the sheath fluid tank following the instructions in the operator's guide.	Refill the sheath tank. See *"Refilling the Sheath Tank" (page 146)*.
Waste Fluid Tank is full. Empty the waste tank following the instructions in the operator's guide.	Replace the waste tank. See *"Emptying/Changing the Waste Tank" (page 148)*.
Ethanol Fluid Tank is empty. Refill the ethanol fluid tank following the instructions in the operator's guide.	Refill the ethanol tank. See *"Refilling the Ethanol Tank" (page 151)*.
Compressed air is not supplied. Confirm if the compressed air hose is connected or the sheath tank is set up properly.	Check that the compressed air supply is supplying rated pressure.
Fluidics cart is not connected. Connect the fluidics cart correctly following the instructions in the operator's guide.	Connect or reconnect the cart connection cable between the main unit and the fluidics cart.
The ambient temperature exceeds the design operating range. Shut down the cytometer.	The cytometer may be overheating. Shut down the cytometer, and allow the temperature to fall within the rated range before rebooting. If the problem persists at temperatures not exceeding 35 °C (95 °F), contact your Sony distributor for service.
The temperature of the sample area is too high.	Shut down the cytometer, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
The temperature of the collection area is too high.	
Sheath leakage is suspected. Check that the fluidics cart is solidly connected and if there is sheath leakage inside the cytometer.	Open the fluidics maintenance door and check for leakages. If the problem persists, contact your Sony distributor for service.
The sheath tank on the fluidics cart is not detected. Check that the sheath tank is attached.	Check that the sheath tank is full and is positioned correctly in the fluidics cart. If the problem persists, contact your Sony distributor for service.
The waste tank on the fluidics cart is not detected. Check that the waste tank is attached.	Check that the waste tank is empty and is positioned correctly in the fluidics cart. If the problem persists, contact your Sony distributor for service.
The temperature control of the sample area failed.	Check that there is no object shielding the base of the cytometer. If the problem persists, contact your Sony distributor for service.
The temperature control of the collection area failed.	
The laser initialization timed out.	The cytometer may be overheating. Shut down the cytometer, and allow the temperature to fall within the rated range before rebooting. If the problem persists at temperatures not exceeding 35 °C (95 °F), contact your Sony distributor for service.

GUI Error Message	Recommended Solution
System power supply error on the PCT board.	Shut down the cytometer, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
AC/DC power supply error on the PCT board.	
250 V power supply error on the HVC board.	
+5 V power supply error on the HVC board.	
-5 V power supply error on the HVC board.	
-12 V power supply error on the HVC board.	
Piezo short error on the HVC board.	
12 V power supply error on the MD1 board.	
24 V power supply error on the MD1 board.	
5 V power supply error on the MD1 board.	
Temperature error from the X axis in the chip stage motor on the MD2 board.	
Temperature error from the Z axis in the chip stage motor on the MD2 board.	
Temperature error from the chip load motor on the MD2 board.	
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12 V power supply error on the MD2 board.	
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5 V power supply error on the MD2 board.	
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24 V power supply error on the MD3 board.	
5 V power supply error on the MD3 board.	
Temperature error from the agitation motor on the MD3 board.	
Pump error on the MD1 board.	
Temperature error from the droplet camera motor on the MD2 board.	The cytometer may be overheating. Shut down the cytometer, and allow the temperature to fall within the rated range before rebooting. If the problem persists at temperatures not exceeding 35 °C (95 °F), contact your Sony distributor for service.
Power supply error on the MEC board.	Shut down the cytometer, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Power supply error on the LCD board.	
Error from FAN1 on the MD1 board.	
Error from FAN2 on the MD1 board.	
Error from FAN3 on the MD1 board.	
Error from FAN on the MD3 board.	
Circuit error on the LD1 board.	
Circuit error on the LD2 board.	
Circuit error on the LD3 board.	
Temperature control of the LD1 failed.	
Temperature control of the LD2 failed.	
Temperature control of the LD3 failed.	
Temperature control of the prism failed.	
Power supply error for LD.	
Power supply error for peltier.	

GUI Error Message	Recommended Solution
Thermistor error for LD1.	Shut down the cytometer, then try again. If the problem persists, contact your Sony distributor for service.
Thermistor error for LD2.	
Thermistor error for LD3.	
Thermistor error for prism.	
Temperature error of the 561nm laser.	The cytometer may be overheating. Shut down the cytometer, and allow the temperature to fall within the rated range before rebooting. If the problem persists at temperatures not exceeding 35 °C (95 °F), contact your Sony distributor for service.
Error on the eeprom.	Shut down the cytometer, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
The ambient temperature is too low.	The cytometer may be too cold. Shut down the cytometer, and allow the temperature to rise within the rated range before rebooting. If the problem persists at temperatures exceeding 10 °C (50 °F), contact your Sony distributor for service.
Communication error on the PCT board.	Shut down the cytometer, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Communication error on the MD1 board.	
Communication error on the MD2 board.	
Communication error on the MD3 board.	
Communication error on the CLM board.	
Communication error on the HVC board.	
No Reply error on the PCT board.	
No Reply error on the MD1 board.	
No Reply error on the MD2 board.	
No Reply error on the MD3 board.	
No Reply error on the CLM board.	
No Reply error on the HVC board.	
Load Chip Error. Wrong side out. Please reinsert the chip.	Insert the chip in the correct orientation, and try loading again. If the problem persists, contact your Sony distributor for service.
Load Chip Error. Up side down. Please reinsert the chip.	
Load Chip Error. Failed to hold the chip. Please reinsert the chip.	
Load Chip Error. Failed to detect the chip. Please reinsert the chip.	
Leakage is suspected in the collection area.	Check the waste fluid line connection to the waste catcher. If the problem persists, contact your Sony distributor for service.
Leakage is suspected in the drip tray.	Inspect the drip tray and check for any fluid leakages. If the problem persists, contact your Sony representative for service.
Failed to start feeding sheath fluid.	• Check that the connectors for all fluidics lines in the fluidics cart and main unit are connected securely and that the lid of the sheath tank is closed tightly. • Check that the black rubber seal is attached to the lid of the sheath tank. • Check that the sorting chip was not inserted back-to-front. • Check that the appropriate pressure is supplied to the instrument. Supply pressure: 500 kPa to 650 kPa (73 psi to 94 psi). If the problem persists, contact your Sony distributor for service.
Failed to fit the chip.	Insert the chip in the correct orientation, and try loading again. If the problem persists, contact your Sony distributor for service.

GUI Error Message	Recommended Solution
Failed to release the chip.	Shut down the cytometer and turn off the compressed air supply. Pull the sample line connector out slightly from the chip loader, and turn the Emergency thumbwheel to eject the chip.
Unload Chip Error. Failed to hold the chip.	
Unload Chip Error. Failed to release the chip.	
The model number is invalid.	Shut down the cytometer, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
The laser configuration is invalid.	
The ADC configuration is invalid.	
Power supply error on the ADC board.	
Power supply error on the ADC2 board.	
Timed out to close the sample door.	
Timed out to control sheath pressure.	
Sheath Fluid Tank is almost empty. Refill the sheath fluid tank following the instructions in the operator's guide.	Refill the sheath tank. See *"Refilling the Sheath Tank" (page 146)*.
Waste Fluid Tank is almost full. Empty the waste tank following the instructions in the operator's guide.	Replace the waste tank. See *"Emptying/Changing the Waste Tank" (page 148)*.
Collection area door is open. Close the collection area door.	Close the sorting area door.
The flip-up door is open. Close the flip-up door.	Close the flip-up door.
Warning for the 561 nm laser.	Laser output does not decrease immediately. The laser can continue to be used, but should be replaced as soon as possible.
Warning for the unsuccessful communication on MD2 board.	This warning does not affect automatic calibration operation, although it may appear during automatic calibration. In this case, continue the automatic calibration process.
Warning for the unsuccessful communication on PCT board.	Shut down the cytometer, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Warning for the unsuccessful communication on MD1 board.	
Warning for the unsuccessful communication on MD3 board.	
Warning for the unsuccessful communication on CLM board.	
Warning for the unsuccessful communication on HVC board.	
The sample loading unit may need to be repaired.	Contact your Sony distributor for scheduled maintenance.
The chip loading unit may need to be repaired.	
The collection loading unit may need to be repaired.	
The pump A may need to be repaired.	
The pump B may need to be repaired.	
The pump C may need to be repaired.	
The e/p regulator for sheath fluid may need to be repaired.	
The e/p regulator for sample fluid may need to be repaired.	
The cooling device for the sample area may need to be repaired.	
The cooling device for the collection area may need to be repaired.	
The sheath filter may need to be repaired.	
LD1 laser power warning.	A reduction in laser output power has been detected. Contact your Sony distributor for advice.
LD2 laser power warning	
LD3 laser power warning	
LD4 laser power warning	
LD1 laser current warning.	
LD2 laser current warning.	
LD3 laser current warning.	

GUI Error Message	Recommended Solution
The LD1 laser is approaching its end of life expiration.	Laser output does not decrease immediately. The laser can continue to be used, but should be replaced as soon as possible.
The LD2 laser is approaching its end of life expiration.	
The LD3 laser is approaching its end of life expiration.	
The LD4 laser is approaching its end of life expiration.	
Failed to record LD1 laser total hours of use.	Total irradiation time was not recorded correctly. The laser is still operational, but you should contact your Sony distributor for service.
Failed to record LD2 laser total hours of use.	
Failed to record LD3 laser total hours of use.	
Failed to record LD4 laser total hours of use.	
DI Water Tank is almost empty. Refill the DI water tank following the instructions in the operator's guide.	Refill the DI water tank. See *"Refilling the DI Water Tank" (page 149)*.
The cytometer has been disconnected. Acquiring and sorting has stopped.	Check the connections to the main unit.
Communication to the cytometer has been lost. Exit and relaunch the software.	Check the connections to the main unit and relaunch SH800 Software.
The initial setup did not complete successfully. Exit and relaunch the software.	Exit SH800 Software and try launching again.
Acquiring and sorting has stopped because available free size of Hard Disk Drive is not enough.	Insufficient remaining space on the hard disk. Delete unnecessary files to increase the available free capacity.
The application is already running. This instance will now exit.	Shut down the SH800 Software instance that is already running.
Failed to save settings.	Try saving the settings again.
Parameter count in a selected file is different from current settings.	Select a file with the same number of parameters as the current settings.
A selected file is wrong format.	Select a file in the correct format.
Unable to maintain stable droplet breakoff.	An error occurred when the control breakoff function was turned on. If a droplet formation problem is detected during measurement, acquisition stops. Try running [Sort Calibration]. If the problem persists, contact your Sony distributor for service.
The selected drive is not external storage device. Please select the external storage device.	Specify an external storage device.
The format of this drive is invalid. Only NTFS format is valid. Please use NTFS formatted drive.	Use only NTFS-formatted drives.
You do not have write permission for the selected folder. Please add write permission to the folder or select another folder with write permission.	You do not have write access privileges for the selected folder. Change the folder access privileges or specify a folder with write access privileges.
Available disk free space is low.	Insufficient remaining space on the disk. Delete unnecessary files to increase the available free capacity.
This chip has been used over the duration limit (12 hours) with the 405 nm laser. Please exchange for a new chip.	Replace the sorting chip.
This chip has been used over the duration limit (12 hours) with the 405 nm laser. You cannot use this chip any longer with the 405 nm laser. Please exchange for a new chip.	
System resources are scarce and may cause the application performance to be worse. Would you like to close unnecessary worksheets?	Close any unnecessary worksheets.
A number of plots are opened and may cause the application performance to be worse. Please close unnecessary plots or worksheets.	
System resources are scarce and may cause the application to shutdown. Would you like to restart this application?	Stop data acquisition and measurement, and restart the SH800 Software.

GUI Error Message	Recommended Solution
System resources are scarce because of multiple applications/processes running in the background. This may impact the performance of the SH800 Software. Please close unnecessary applications/processes.	Close any unnecessary running applications and processes.
Invalid biexponential parameters were selected.	Specify valid biexponential parameters.
Cannot assign this tube because available free size of HDD is not enough. Please exceed available free size space of HDD the hard drive.	Cannot assign due to insufficient remaining space on HDD. Delete unnecessary files to increase the available free capacity.
Detected UNKNOWN state. Please check the cytometer SH800.	Check the cytometer for any abnormalities.
Unable to maintain stable droplet breakoff. The measurement will be stopped and Control Breakoff will be disabled.	Data acquisition stops. Check the instrument settings, as required.
This chip has been used over 9 hours with the 405 nm laser; the limit with a 405 nm laser is 12 hours. Please exchange the chip before the 12 hour limit.	The sorting chip is approaching the end of its service life. Replace the sorting chip.
Cannot receive droplet camera image continuously. If you have some trouble, please stop acquisition.	The droplet camera image cannot be acquired. If a problem occurs, stop data acquisition.
Cannot receive side stream camera image continuously. If you have some trouble, please stop acquisition.	The side stream monitor image cannot be acquired. If a problem occurs, stop data acquisition.
The droplet condition has changed since the last Sort Calibration. The Sort Calibration should be performed again for stable sorting.	The formation of droplets has changed since auto calibration was last executed. Run [Sort Calibration] again to ensure stable sorting conditions. Use only within the specified temperature range. Do not use under conditions where the temperature is subject to rapid changes.
Failed to initialize the sheath pressure regulator.Sheath pressure is too high. Please restart the cytometer and software. If the error reoccurs, please contact your service representative.	Shut down the main unit, and wait a while before trying again. If the problem persists, contact your Sony distributor for service.
Failed to initialize the sample pressure regulator. Sample pressure is too high. Please restart the cytometer and software.If the error reoccurs, please contact your service representative.	
High sheath pressure is detected while initializing the regulator. Please contact your service representative.	An unusual pressure value was detected. You can continue with operations, but you should contact your Sony distributor for service.
High sample pressure is detected while initializing the regulator. Please contact your service representative.	
Low sheath pressure is detected while initializing the regulator. Please contact your service representative.	
Low sample pressure is detected while initializing the regulator. Please contact your service representative.	
Failed to move the waste tray.	The waste fluid tray is not operating correctly. Contact your Sony distributor for service.
Failed to agitate the sample tube.	The agitation unit of the sample loader is not operating correctly. Contact your Sony distributor for service.
Sorting error has been detected. Acquisition was stopped.	A sorting error has occurred. Check that there are no abnormal objects in the collection area. Wait for a short while before restarting sorting. If the problem persists, contact your Sony distributor for service.
Failed to collection load!!	Loading of the collection stage failed. Check that there are no abnormal objects in the collection area. Wait for a short while before loading again. If the problem persists, contact your Sony distributor for service.

Optional Accessories

For details about purchasing optional accessories, contact your Sony distributor.

Part Name	Part Number
Sorting chip (100 µm)	LE-C3110
Sorting chip (130 µm)	LE-C3113
Cleaning chip	LE-C3100
Automatic setup beads	LE-B3001
6-peak beads	LE-AE700520
Sample line	LE-U3F001A
Sample probe	LE-U3F002
PEEK sample line	LE-U3F021
Probe adapter	LE-U3F023
Sheath filter	LE-U3F003
Sheath filter bypass line (yellow)	LE-U3F004A
DI water filter bypass line (green)	LE-U3F020
DI water tank kit (with DI water tank air filter)	LE-U3F005
Sheath tank	LE-U3F007
Sintered sheath filter	LE-U3F008
Waste tank	LE-U3F009
Waste tank air filter	LE-U3F011A
Ethanol tank kit (with ethanol tank air filter)	LE-U3F012
Air filter for ethanol and DI water tanks	LE-U3F014
DI water tank air filter	LE-U3F019
Pressurized air line connection	LE-U3F016
Waste line connection	LE-U3F017
Ethanol line connection	LE-U3F018
Sheath line connection	LE-U3F022
Sample loader O-ring	LE-U3M001
Deflection plate kit	LE-U3M006
Waste catcher	LE-U3M008
Waste catcher O-ring	LE-U3M009
Rubber packing for sorting area door	LE-U3M012
Fluorescent protein filters	LE-U3L001
Splash guard	LE-U3M013
Slide glass holder (for SH800)	LE-A3101
Slide glass holder (for SH800Z)	LE-A3101A
8-well strips holder (for SH800)	LE-A3102
8-well strips holder (for SH800Z)	LE-A3102A
96-well plate holder	LE-A3103
96-well PCR plate holder	LE-A3104
384-well plate holder kit	LE-K3103
384-well plate holder	LE-A3107
384-well PCR adapter	LE-A3108
AMS evacuator connection kit	LE-A3105

Software License

LGPL

This product uses the following LGPL software. The customer is entitled to obtain, modify, and redistribute the source code for the software.

DirectShow.NET

Source codes can be obtained from the "\Others\OSS\SourceCode" folder on the supplied installation disc.
However, Sony cannot accept inquiries regarding the content of these source codes.
For license information, refer to the "\Others\License" folder on the supplied installation disc.

Apache License

This product uses the Apache license software, ZXing.
For license information, refer to the "\Others\License" folder on the supplied installation disc.

Microsoft Public License (Ms-PL)

This product uses the Microsoft Public License (Ms-PL) software, WPF Toolkit, DotNetZip, and Fluent Ribbon Control Suite.
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Disposal of Old Electrical & Electronic Equipment (Applicable in the European Union and other European countries with separate collection systems)

This symbol on the product or on its packaging indicates that this product shall not be treated as household waste. Instead it shall be handed over to the applicable collection point for the recycling of electrical and electronic equipment. By ensuring this product is disposed of correctly, you will help prevent potential negative consequences for the environment and human health, which could otherwise be caused by inappropriate waste handling of this product. The recycling of materials will help to conserve natural resources. For more detailed information about recycling of this product, please contact your local Civic Office, your household waste disposal service or the shop where you purchased the product.

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